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(57) Abstract

A solid-phase nucleotide synthesis intermediate useful for chemical synthesis of oligodeoxyribonucleotides and oligoribonucleotides comprises: (1) a particulate support comprising a porous polymer whose backbone comprises optionally substituted acrylate or methacrylate moieties; (2) a nucleoside; and (3) a linker having a first and a second end, the first end being covalently attached to the particulate support and the second end being covalently attached to the nucleoside, the linker spacing the nucleoside at least 3 atoms away from the polymer. The linker can comprise at least one optionally substituted aliphatic diamine. Alternatively, the linker can comprise a polyethylene glycol moiety. Preferably, the porous polymer is a methacrylate-vinylidene polymer. The solid-phase support thus produced can be used for oligodeoxyribonucleotide synthesis by either the phosphite-triester or the phosphotriester processes.

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DERIVATIZED ORGANIC SOLID SUPPORT FOR NUCLEIC ACID SYNTHESIS

BACKGROUND

This invention is directed to solid phase supports for oligodeoxyribonucleotide and oligoribonucleotide synthesis and methods for their use.

The ability to synthesize specific DNA and RNA sequences quickly and easily has had an impact on molecular biology unmatched by few other techniques. Such synthesized sequences are frequently used for hybridization probes, linkers for gene cloning, primers for nucleic acid amplification reactions such as the polymerase chain reaction (PCR) and the ligase chain reaction (LCR), and primers for nucleic acid sequencing.

DNA and RNA are both long polymeric molecules comprised of monomers known as nucleotides. Each nucleotide is composed of a nitrogenous organic base, a five-carbon sugar, and a phosphate residue. The chemical combination of the base and the sugar, which can exist separately, is known as a nucleoside. In DNA, the sugar is deoxyribose, while in RNA, it is ribose, which contains one more oxygen atom. In DNA, the bases are normally adenine (A), guanine (G), cytosine (C), and thymine (T), while in RNA, the bases are normally adenine, guanine, cytosine, and uracil (U). In natural DNA and RNA, there can be thousands of nucleotide units in one polymer, known generically as a polynucleotide: polydeoxyribonucleotide for DNA and polyribonucleotide for RNA. Shorter chains of DNA and RNA can also exist, either as a result of chemical synthesis or breakdown of a larger chain. These are known generically as oligonucleotides:

oligodeoxyribonucleotides for DNA and oligoribonucleotides for RNA.

Natural DNA consists of two strands of polydeoxyribonucleotides bound together. The strands are held together by base pairing, so that an adenine always pairs with a thymine, and a cytosine always pairs with a guanine. Although RNA generally occurs as a single stranded polyribonucleotide, it also can form double strands by the same base pairing rules, with U behaving in the same way as T in DNA. The specificity of base pairing means that synthesis of DNA in the laboratory must be extremely accurate to be useful, as the insertion of even one wrong base would disrupt the structure and make the resulting product useless.

Oligodeoxyribonucleotide synthesis in the laboratory is typically carried out by one of two reaction sequences: the phosphotriester approach and the phosphite triester approach. The general reaction sequences are described in "Oligonucleotide Synthesis: A Practical Approach," M.J. Gait, ed., IRL Press, Oxford, 1984, incorporated herein by this reference. In either case, the reaction sequence starts with a 5'-protected nucleoside bound to a solid-phase support through the 3'-carbon of the sugar. After removal of the protecting group from the 5'-protected nucleoside, a 5'-OH moiety is available for reaction with the 3'-position of either a phosphotriester or a phosphite triester activated nucleoside (also referred to as "activated intermediates").

The solid support plays a crucial role in the success of either reaction scheme. The solid support must not interfere with or contaminate the reaction and must make the nucleosides attached to it available for reaction with the activated intermediates. The choice of solid support is

important in the maintenance of a high repetitive yield, which is an important factor in the efficient synthesis of relatively long oligodeoxyribonucleotides.

Currently, a widely-used solid support is the inorganic material controlled pore glass (CPG). The use of this support is described in U.S. Patent No. 4,458,066 to Caruthers et al., incorporated herein by this reference. Although CPG has proven useful, it has some drawbacks. CPG does not permit high levels of nucleotide loading and can be responsible for spurious indications of coupling yields. Additionally, CPG is susceptible to degradation by reagents used in the deprotection step of synthesis, such as concentrated ammonia and trichloroacetic acid, and this can lead to contamination of the product. Finally, CPG can support chain growth on its surface at sites other than those associated with the 5'-terminus of an attached nucleotide and thus give rise to extraneous protected products.

Because of these drawbacks, in some applications it may be desirable to use an organic support. The use of an organic support would be desirable in order to provide a support compatible with a wide variety of commercially available nucleotide synthesis apparatus while providing efficient synthesis at lower cost than can be obtained with CPG. It would additionally be desirable for the user to be able to attach a variety of spacers of different yet defined length for optimal performance of the synthesis.

However, despite this recognized need, previously-used organic supports for solid-phase nucleotide synthesis have not proven adequate for a variety of reasons including slow diffusion rates of activated nucleotides into the support, excessive swelling of the support, irreversible

absorption of reagents onto the support, low nucleotide loading, and poor repetitive yields.

Therefore, there is a need for an improved organic support for solid-phase nucleotide synthesis that can provide superior nucleotide loading, high repetitive yields, stability with commonly-used nucleotide synthesis apparatus, at a cost lower than presently-available supports.

SUMMARY

We have invented novel supports for oligonucleotide synthesis that meet these needs. With the invention thereof, processes for producing such supports and methods for the synthesis of nucleic acids have also been invented.

In general, a solid-phase support, according to the present invention, referred to as a "nucleotide synthesis intermediate," comprises:

(1) a particulate support comprising a porous polymer whose backbone comprises optionally substituted acrylate or methacrylate moieties;

(2) a nucleoside; and

(3) a linker having a first and a second end, the first end being covalently attached to the particulate support and the second end being covalently attached to the nucleoside, the linker spacing the nucleoside at least 4 atoms away from the polymer.

Preferably, the nucleoside is a protected deoxyribonucleoside.

Preferably, the porous polymer is a methacrylate-vinylidene copolymer.

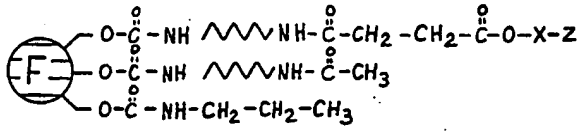
In a preferred embodiment, the linker comprises at least one aliphatic diamine. Each aliphatic diamine optionally contains up to 2 heteroatoms replacing carbon atoms and selected from the group consisting of O and N. The heteroatoms are bonded solely to carbon and are separated from the terminal amino group of the diamine by at least one carbon atom. The aliphatic diamines have a total chain length of from about 3 to about 36 atoms, with the proviso that, if more than one diamine is present in the linker, the diamines are linked to each other by carbonyl groups.

If the linker comprises at least one aliphatic diamine, it can be attached to the porous polymer by a carbamate moiety at one end of the diamine. The nucleoside is then linked to the other end of the diamine, preferably by a succinyl ester group.

Alternatively, a linker comprising at least one aliphatic diamine can be linked attached to the porous polymer by the structure $-CH_2-CH(OCOCH_3)-CH_2-$. In this structure, the first amino group of the diamine moiety attached to the particulate support is substituted with an acetyl group.

As another alternative, the linker can comprise a polyethylene glycol moiety containing from about 3 to about 10 ethylene glycol monomers, preferably from about 4 to about 7 ethylene glycol monomers. In this alternative, the polyethylene glycol moiety has a first end and a second end, with the first end covalently attached to the particulate support, and the second end terminating in an amine moiety. The nucleoside is linked to the amine moiety at the second end of the polyethylene glycol linker by an amide linkage between the amine moiety and a carboxyl group in covalent linkage with the nucleoside.

Particular nucleotide synthesis intermediates according to the present invention include those shown below as Formulas I-VI. In these formulas, (F) is a macroreticular methacrylate-vinylidene polymer, X is a nucleoside, and Z is a 5'-protecting group.



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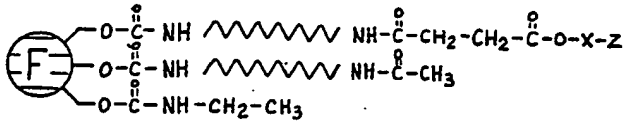

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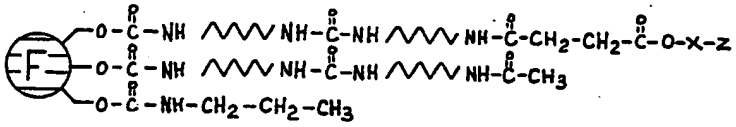

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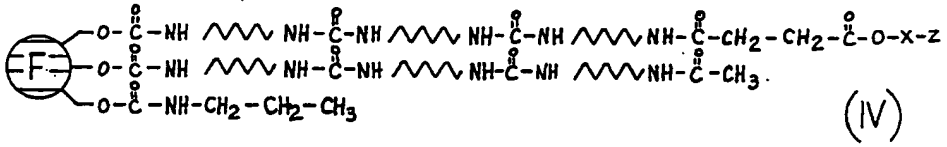

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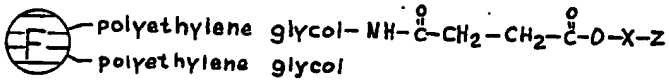

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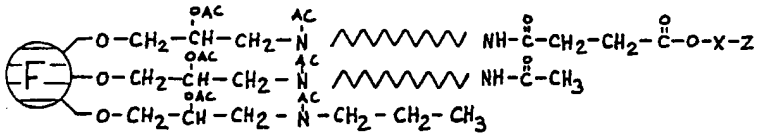


(VI)

A process of producing nucleotide synthesis intermediates according to the invention comprises generally:

(1) coupling:

(a) a particulate support comprising a porous polymer whose backbone comprises optionally substituted acrylate or methacrylate moieties, with:

(b) a linker having a first and a second end, the first end being covalently attached to the particulate support; and

(2) coupling the second end of the linker to a nucleoside, the linker spacing the nucleoside at least 4 atoms away from the polymer.

Nucleotide synthesis intermediates according to the present invention can be used for nucleotide synthesis in either the phosphite-triester or the phosphotriester synthesis method.

In general, the phosphite-triester method for synthesis of DNA, used with solid supports according to the present invention, comprises the steps of selecting a solid-phase intermediate containing a 5'-protected deoxyribonucleoside, removing the protecting group, coupling the free 5'-OH group with a phosphoramidite, oxidizing the phosphite triester linkage to a phosphotriester linkage,

acetylating unreacted 5'-OH groups, and cleaving the synthesized oligonucleotide from the solid support.

In general, the phosphotriester method for synthesis of DNA, used with solid supports according to the present invention, comprises the steps of selecting a solid-phase intermediate containing a 5'-protected deoxyribonucleoside, removing the protecting group, coupling a phosphorylated nucleotide to form a dinucleotide, and cleaving the synthesized oligonucleotide from the solid support.

Nucleotide synthesis intermediates according to the present invention are capable of supporting efficient synthesis of nucleotides up to at least 1000 bases in length.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description, appended claims, and the accompanying drawings where:

Figure 1 is a capillary gel electropherogram of a 24-base oligodeoxyribonucleotide synthesized using controlled pore glass;

Figure 2 is a capillary gel electropherogram of the same 24-base oligodeoxyribonucleotide synthesized using a solid support according to the present invention;

Figure 3 is a capillary gel electropherogram of the same 24-base oligodeoxyribonucleotide synthesized using a different solid support according to the present invention;

Figure 4 is a capillary gel electropherogram of a 35-base oligodeoxyribonucleotide synthesized using controlled pore glass;

Figure 5 is a capillary gel electropherogram of the same 35-base oligodeoxyribonucleotide synthesized using a solid support according to the present invention;

Figure 6 is a capillary gel electropherogram of the same 35-base oligodeoxyribonucleotide synthesized using a different solid support according to the present invention;

Figure 7 is a capillary gel electropherogram of the same 35-base oligodeoxyribonucleotide synthesized using another different support according to the present invention;

Figure 8 is a capillary gel electropherogram of the same 35-base oligodeoxyribonucleotide synthesized using another different support according to the present invention;

Figure 9 is a slab gel electropherogram of 24- and 35-base oligodeoxyribonucleotides synthesized with different solid supports according to the present invention, using different nucleotide synthesis apparatuses;

Figure 10 is a slab gel electropherogram of 35-base oligodeoxyribonucleotides synthesized with different solid supports according to the present invention, using different nucleotide synthesis apparatuses;

Figure 11 is a slab gel electropherogram of 35- and 51-base oligodeoxyribonucleotides synthesized with different solid supports according to the present invention, using different nucleotide synthesis apparatuses;

Figure 12 is a slab gel electropherogram of 51-, 70-, and 101-base oligodeoxyribonucleotides synthesized with different solid supports according to the present invention, using different nucleotide synthesis apparatuses;

Figure 13 is a slab gel electropherogram of 24- and 35-base oligodeoxyribonucleotides synthesized with different solid supports according to the present invention, using different nucleotide synthesis apparatuses, showing different syntheses than depicted in Figure 11;

Figure 14 is a slab gel electropherogram of 35-base oligodeoxyribonucleotides synthesized with different solid supports according to the present invention, using different nucleotide synthesis apparatuses, showing different syntheses than depicted in Figure 10;

Figure 15 is a slab gel electropherogram of 51-base oligodeoxyribonucleotides synthesized with different solid supports according to the present invention, using different nucleotide synthesis apparatuses, showing different syntheses than depicted in Figures 10 and 14;

Figure 16 is a slab gel electropherogram of 51-base oligodeoxyribonucleotides synthesized with different solid supports according to the present invention, using different nucleotide synthesis apparatuses;

Figure 17 is a slab gel electropherogram of 101-base oligodeoxyribonucleotides synthesized with different solid supports according to the present invention;

Figure 18 is a slab gel electropherogram of 35-, 70-, and 101-base oligodeoxyribonucleotides synthesized with different solid supports according to the present invention;

Figure 19 is a slab gel electropherogram of products from a polymerase chain reaction synthesis using a 17-base oligodeoxyribonucleotide produced with a solid support according to the present invention as a primer; and

Figure 20 is a gel showing the result of a chain-terminating DNA sequencing reaction performed on the products from the polymerase chain reaction synthesis.

DEFINITIONS

Definitions for a number of terms that are used in the following Descriptions, Examples, and appended claims are collected here for convenience.

"Polymer": A polymer is a molecule comprised of a number of individual monomer units chemically linked together. The individual monomer units can be the same or different. A polymer whose individual monomer units are carbon-containing is an organic polymer, while a polymer whose individual monomer units do not contain carbon is an inorganic polymer.

"Partially Hydrophilic": A polymer is partially hydrophilic when at least about 25% of the exposed individual monomer units can interact with water.

"Polar": A solvent is polar when it can mix with water and has molecules which are polarized to produce partial positive and negative electrical charges at opposite ends of the molecule.

"Aprotic": A solvent is aprotic when it has no proton that can be removed by the action of a base in an aqueous solution.

"Optionally Substituted": A polymer or other molecule is optionally substituted when it can contain substituent groups in place of hydrogen atoms on the polymer. When this term is used, both the molecule without the substituent groups and the molecule with the substituent groups are included. The optional substituents are of a type that do not alter the overall chemical reactivity of the polymer or other molecule.

"Aliphatic": An aliphatic molecule is an organic molecule whose backbone contains carbon and hydrogen with no carbon-carbon double bonds or triple bonds.

"Diamine": An organic molecule containing two amine ($-NH_2$) groups, typically at opposite ends of the chain. As used herein, the term "diamine" includes derivatives of aliphatic diamines optionally containing up to 2 heteroatoms. In such derivatives, the heteroatoms replace carbon atoms.

"Porous": A polymer is porous when it contains pores that can be penetrated by a solvent into which the polymer is placed.

"Mean Diameter": The arithmetic average of the diameters of an assemblage of particles.

"Monoderivatized": A molecule is monoderivatized when it contains one substituent of a particular type, as distinguished from two or more substituents.

"Acylating": Reacting an alcohol or amino group with a reagent containing an acyl (RCO-) group. A common example of acylation is acetylation, in which R is methyl (CH_3).

"Capping": A process for ensuring that hydroxy (-OH) residues remaining on a polymer are blocked, typically by forming an ester linkage.

"Spacer" or "Linker": These terms, used interchangeably, both refer to a portion of a composite structure that spatially separates a reactive molecule from a large polymer so that reagents can react readily with the reactive molecule without interference from the polymer.

"Activated Nucleoside": A nucleoside one of whose 3'- or 5'- positions is substituted with a group that can

react to form a bond with another nucleoside is known as "activated." Typically, an activated nucleoside incorporates an acid group such as an ester function.

"3'-Protected" and "5'-Protected": Because the 3'- and 5'-positions of the sugar residue of the nucleotide are involved in the formation of the phosphodiester bonds, each nucleotide to be added must be protected on one of these groups to ensure that only the correct position of the sugar residue reacts to form the phosphodiester bond.

"Exocyclic Amino Protecting Group": Certain of the bases of the nucleotides, in particular adenine, guanine, and cytosine, have amino groups whose reactivity could interfere with oligodeoxyribonucleotide synthesis. These amino groups must be blocked or protected, typically by acylation. The group used for protection is referred to as an exocyclic amino protecting group.

"Nucleotide Synthesis Intermediate": A substituted polymer according to the present invention incorporating an activated nucleotide and suitable for oligonucleotide synthesis is designated a nucleotide synthesis intermediate.

DESCRIPTION

We have discovered that a number of organic polymers not based on dextran or other carbohydrate polymers are useful as solid supports for solid-phase oligodeoxyribonucleotide synthesis.

I. THE POLYMERS

The polymers useful for synthesis according to the present invention are porous organic polymers, at least partially hydrophilic and having hydroxy residues, whose backbone comprises optionally substituted acrylate or methacrylate moieties. Preferably, the backbone comprises straight-chain acrylate or methacrylate moieties. The optional substitutions can include any groups that do not significantly react with any of the other reagents employed for coupling or subsequent DNA synthesis, such as 1,1'-carbonyldiimidazole or oxidizing agents. Such groups include, but are not limited to, aryl groups, alkoxy groups, and heterocyclic groups. The polymers are substantially free of pyranose or furanose moieties or their derivatives.

Specific and preferred polymers that fall within the description include, but are not limited to:

(1) Macroreticulated methacrylate-vinylidene copolymers available from Tosohaas (Philadelphia, Pennsylvania) under the name of TOYOPEARL™ and from Merck (Darmstadt, Germany) under the name of FRACTOGEL™, described by U.S. Patents Nos. 4,224,415; 4,256,840; 4,297,220; and 4,501,816 to Meitzner et al., all of which are incorporated herein by this reference;

(2) Homopolymers of pentaerythritol dimethacrylate, described generally in U.S. Patent No. 4,246,362 to Sasaki et al., incorporated herein by this reference;

(3) Copolymers of pentaerythritol dimethacrylate and a methacrylate monomer, described by U.S. Patent No. 4,246,362 to Sasaki et al., supra;

(4) Copolymers of a hydrophilic monomer selected from the group consisting of hydroxyalkyl methacrylates, aminoalkyl methacrylates, N-vinylpyrrolidone, acrylonitrile,

methacrylonitrile, acrylic acid, methacrylic acid, and mixtures thereof, with a substantially hydrophobic monomer selected from the group consisting of ethylene dimethacrylate, ethylene diacrylate, methylenebisacrylamide, diethylene glycol methacrylamide, poly (ethyleneglycol) methacrylamide, neopentyl glycol diacrylate, neopentyl glycol dimethacrylate, trimethylol propane trimethacrylate, divinylbenzene, and mixtures thereof, described generally in U.S. Patent No. 4,184,020 to Lim et al., incorporated herein by this reference;

(5) Copolymers of polar monomers such as hydroxyalkyl acrylates and hydroxalkyl methacrylates, with non-polar monomers such as alkyl acrylates and methacrylates, together with cross-linking agents such as alkylene diacrylates and methacrylates, described generally in U.S. Patent No. 4,135,892 to Coupek et al., incorporated herein by this reference; and

(6) Polymers comprising the reaction product of:

(a) a hydroxyalkyl or aminoalkyl acrylate or methacrylate, and acrylamide or methacrylamide;

(b) an alkylene diacrylate or dimethacrylate, or an acrylate or methacrylate of a polyalcohol, a dihydroxy or polyhydroxy ether, an alkylene bisacrylamide, or divinylbenzene; and

(c) acrylanilide, methacrylanilide, phenyl acrylate, phenyl methacrylate, phenoxyalkyl acrylate, phenoxylalkyl methacrylate, or styrene, with the aromatic ring being chloromethylated, as described generally in U.S. Patent No. 3,925,267 to Coupek et al, incorporated herein by this reference.

Preferred organic polymers are the macroreticulated methacrylate-vinylidene copolymers.

As described below, in a particularly preferred embodiment, the polymers are either extended with alkanediamine spacers or derivatized with polyethylene glycol to provide a framework for attachment of the protected deoxyribonucleoside. Alternatively, it is also possible to directly attach the protected deoxyribonucleoside to the hydroxy groups of the polymer.

In general, the nucleotide synthesis intermediate produced by this process comprises:

(1) a particulate support comprising a porous polymer whose backbone comprises optionally substituted acrylate or methacrylate moieties;

(2) a nucleoside; and

(3) a linker having a first and a second end, the first end being covalently attached to the particulate support and the second end being covalently attached to the nucleoside, the linker spacing the protected deoxyribonucleoside at least 4 atoms away from the polymer.

In this structure, the linker is used to separate the first nucleoside from the support, so that the nucleoside is reactive to form a bond with a second nucleoside, thus beginning the process of oligonucleotide synthesis.

II. PROCESS FOR PRODUCING A DERIVATIZED SOLID-PHASE SUPPORT

The organic polymers defined and disclosed herein can be used in a process for producing a particulate porous organic solid-phase support derivatized with, e.g., a protected deoxyribonucleoside.

Generally, this process involves:(1) attaching one end of the spacer to the polymer; (2) attaching the nucleoside

to the other end of the spacer so that the nucleoside is separated from the polymer by the spacer; and (3) blocking any reactive groups on the polymer that might interfere with subsequent nucleotide synthesis reactions.

A. Support with Alkanediamine Spacer

One version of the process results in a support incorporating an alkanediamine spacer.

In general, this process comprises the steps of:

(1) coupling:

(a) a porous solid-phase particulate support comprising a porous polymer, at least partially hydrophilic and having hydroxy residues, whose backbone comprises straight-chain optionally substituted acrylate or methacrylate moieties, the porous polymer being extended with a linear polymer having first and second ends, the linear polymer being covalently linked through the first end to the porous polymer, the linear polymer comprising at least one aliphatic diamine, the linear polymer having an amino group at its second end, with:

(b) a nucleoside, such that the nucleoside is covalently linked to the amino group at the second end of the linear polymer;

(2) acylating terminal amino groups remaining uncoupled; and

(3) blocking hydroxy residues on the solid phase support.

Each aliphatic diamine can optionally contain up to 2 heteroatoms replacing carbon atoms. The heteroatoms are selected from the group consisting of oxygen and nitrogen. The heteroatoms are bonded solely to carbon and are separated

from the terminal amino group of the diamine by at least one carbon atom.

The aliphatic diamines have a total chain length of from about 3 to about 36 carbon atoms. If more than one diamine is present in the linker, the diamines are linked to each other by carbonyl groups.

In general, the nucleotide synthesis intermediate produced by this process comprises:

(1) a porous solid-phase particulate support comprising a porous organic polymer, at least partially hydrophilic and having hydroxy residues, whose backbone comprises straight-chain optionally substituted acrylate or methacrylate moieties;

(2) a linear polymer having first and second ends, the linear polymer being covalently linked through the first end to the particulate support, the linear polymer comprising at least one aliphatic diamine as described above, the linear polymer having an amino group at its second end available for reaction with an activated protected deoxyribonucleoside, the linkage between the particulate support and the first end of the linear polymer comprising a carbamate moiety; and

(3) a nucleoside linked to the second end of the linear polymer.

1. The Condensation Step

This step involves the condensation or coupling of at least one alkanediamine moiety to the polymer to produce a polymer extended with a spacer or linker. The spacer or linker is subsequently coupled to a nucleoside, resulting in the nucleotide synthesis intermediate.

(a) Preferred Polymers

Preferred polymers for assembly of the solid-phase support are the macroreticulated methacrylate-vinylidene copolymers TOYOPEARL™ (available from Tosohaas) and as FRACTOGEL™ (available from Merck). Particularly preferred polymers are such methacrylate-vinylidene copolymers having particles of from about 5 microns to about 200 microns in mean diameter, preferably from about 30 microns to about 70 microns; most preferred are particles having a mean diameter of about 45 microns. These values are mean values, and individual particles vary somewhat in size.

These preferred polymers can have a range of porosities; most preferred are polymers having pores of a size that excludes proteins of molecular weight greater than $5x10^6$. Also particularly preferred are polymers having pores of a size that excludes proteins of a molecular weight greater than about $5x10^7$.

(b) Diamines

As used herein, the term "diamine" includes derivatives of aliphatic diamines optionally containing up to 2 heteroatoms. In such derivatives, the heteroatoms replace carbon atoms. The heteroatoms are selected from the group

consisting of oxygen and nitrogen. The heteroatoms are bonded solely to carbon and are separated from the terminal amino group of the diamine by at least one carbon atom.

The diamines can be either straight-chain or branched-chain. Preferably, the diamines are straight-chain.

The diamine linker can have a total chain length, counting only methylene ($-CH_2-$) groups and heteroatoms substituting for carbon and not amino or carbonyl groups, of from 3 to 36 atoms, more preferably 6 to 18 carbon atoms. Each diamine moiety preferably has a chain length of from 3 to 12 atoms. If there is more than one diamine moiety, the diamine moieties can have the same or different chain lengths. If branched-chain diamines are included, the chain length intended is the longest continuous chain present.

Particularly preferred diamine arrangements are the following:

(1) One diamine moiety of six carbon atoms;

(2) One diamine moiety of 12 carbon atoms;

(3) Two diamine moieties of six carbon atoms each; and

(4) Three diamine moieties of six carbon atoms each.

(c) Reaction Conditions

The coupling reaction is preferably performed in a polar, aprotic solvent such as acetonitrile. The polymer to be derivatized is suspended in the solvent at a concentration of from about 0.05-0.2 g/ml of solvent. Preferably the polymer is suspended at about 0.1 g/ml of solvent. The coupling reaction is performed using a coupling agent.

Preferably, the coupling agent is 1,1'-carbonyldiimidazole, and is used at a concentration of about 1.0 mole/l. Other coupling agents, such as carbodiimides, are also well known in the art.

The coupling reaction results in the coupling of some of the hydroxy residues of the polymer to diamines. The hydroxy residues not coupled to diamines are blocked, as described below, to prevent interference with the remaining reactions.

Accordingly, the first stage in a preferred coupling reaction is the reaction of 1,1'-carbonyldiimidazole, at a concentration of about 1.0 mole/l, with the polymer suspended in acetonitrile at about 0.1 g/ml. This reaction occurs at a temperature of about 15°-30°C, preferably at room temperature. Preferably, the reaction is allowed to proceed from about 2-6 hours, more preferably for about 4 hours. After this reaction is substantially complete, the polymer can be used for coupling to a diamine.

The first alkanediamine to be coupled to the polymer is then added to the reaction mixture to a concentration of about 0.5-2 mole/l, preferably at about 1.0 mole/l. The reaction is allowed to occur overnight.

Subsequent to the coupling of the alkanediamine to the polymer, the remaining hydroxy-containing sites on the polymer are blocked by the addition of a quantity of an aliphatic monoamine sufficient to react with all remaining activated hydroxyl groups. Preferably, the aliphatic monoamine is selected from the group consisting of ethylamine, n-propylamine, n-butylamine, and piperidine. Most preferably, the aliphatic monoamine is n-propylamine. Most preferably,

the blocking reaction is carried out prior to the coupling of additional alkanediamine residues, if any.

If more alkanediamine residues are to be coupled to the polymer, the blocked, monoderivatized polymer is suspended in a halogen-containing solvent such as dichloromethane. The coupling reagent p-nitrophenylchloroformate is then added to a concentration of about 0.5-2 mole/l, preferably about 1 mole/l. Then a methylated pyridine derivative, serving as a base, is added to a concentration of about 0.5 to about 3.0 moles/l, preferably about 1.5 moles/l. Preferably, the methylated pyridine derivative is collidine. The reaction of the monoderivatized polymer is allowed to occur overnight at a temperature between about 15°C and 30°C, preferably room temperature.

Alternatively, a second alkanediamine residue can be added by means of reaction with carbonyldiimidazole. The monoderivatized solid support is suspended in dry acetonitrile, 1,1'-carbonyldiimidazole is added to a concentration of 0.5 mole/l to about 1.5 mole/l, preferably about 1.0 mole/l, and the reaction allowed to proceed at a temperature of 15°-30°C, preferably room temperature, for a reaction time of about 2 hours to about 6 hours, preferably about 4 hours.

The support is then filtered and washed with acetonitrile, and then resuspended in that solvent. The second alkanediamine to be coupled is added at a concentration of about 0.5 mole/l to about 1.5 mole/l, preferably about 1.0 mole/l, and the mixture shaken overnight. The diderivatized support is washed extensively. A preferable washing routine is 10 times with acetonitrile, 20 times with distilled water, 3 times with acetonitrile, 3 times with dichloromethane, and

twice with ether. The support is then dried under vacuum to remove the remaining ether.

2. The Coupling Step

The nucleoside coupled is preferably an activated aryl ester of a protected deoxyribonucleoside. In the ester, the carboxyl function is being sufficiently activated to create a linkage between the deoxyribonucleoside and the solid support.

(a) The Protected Nucleoside

The process of the present invention can be used to produce nucleotide synthesis intermediates containing either a deoxyribonucleoside, for DNA synthesis, or a ribonucleoside, for RNA synthesis. Additionally, the nucleoside can be protected at either its 5'-end or its 3'-end. Typically, the nucleoside is protected at the 5'-end, for synthesis in the 3'- to 5'- direction. However, the nucleoside can also be protected at the 3'-end, for synthesis in the 5'- to 3'- direction.

Protected deoxyribonucleosides are commonly used for coupling to the solid support. Protection of both the exocyclic amino groups of adenine, cytosine, and guanine, is well understood in the art. Thymine and uracil, which have no exocyclic amino group on its base, does not require a protecting group thereon. Protection of either the 5'-hydroxyl group or 3'-hydroxyl group of all of the four common deoxyribonucleosides and the four common ribonucleosides is also well understood in the art.

Typically, the exocyclic amino groups are protected by acylation. The benzoyl group is used to protect both adenine and cytosine, while the isobutyryl group is used to protect guanine. Other alternative protecting groups are known, such as phthaloyl or di-N-butylaminomethylene for the amino group of adenine.

As those in the art appreciate, the 5'-hydroxyl or the 3'-hydroxyl group is typically protected with triphenylmethyl (trityl), 4-methoxytriphenylmethyl (methoxytrityl), or 4,4'-dimethoxytriphenylmethyl (dimethoxytrityl). The dimethoxytrityl protecting group is generally preferred.

Preferably, the protected deoxyribonucleoside used in the coupling reaction is protected at the 5'-hydroxyl group.

(b) The Coupling Reaction

The coupling of the activated aryl ester of the protected deoxyribonucleoside with the support typically occurs in two stages: (i) formation of the activated aryl ester from a carboxyl-containing derivative of the protected nucleotide; and (ii) reaction of the activated aryl ester with the amino-terminated derivatized support. Other coupling reactions are possible that result in the coupling of the protected deoxyribonucleoside to the amino terminus of the support. See, for example, M.J. Gait, "Oligonucleotide Synthesis: A Practical Approach," supra.

The activated aryl ester is preferably an ester of a succinyl or oxalyl derivative of the protected nucleoside with a phenol substituted with at least one electron-withdrawing substituent. Such a substituent activates the derivative for

reaction with an electron-rich group like a hydroxyl group. Most preferably, the activated aryl ester is a succinyl derivative and the substituted phenol is p-nitrophenyl. Therefore, the compound coupled is most preferably a p-nitrophenyl ester of a succinylated protected nucleoside.

A preferred coupling procedure is described below and sets forth in detail the coupling of the p-nitrophenyl ester of succinylated 5'-protected thymidine. It is to be understood that other nucleosides, including ribonucleosides, can be coupled in much the same manner; any variations are considered to be within the skill of the art.

Succinylated 5'-protected thymidine is dissolved in dioxane at a concentration of about 0.113 mole/l, containing pyridine (about 0.045% (v/v)), and p-nitrophenol is added at about 10:1 molar ratio to the succinylated thymidine. The coupling agent, 1,3-dicyclohexylcarbodiimide, is then added at about a 2.5:1 molar ratio to the succinylated thymidine. The coupling reaction is allowed to go to completion (typically about three hours), and dicyclohexylurea precipitates out. The supernatant, containing the p-nitrophenyl ester, is added to the derivatized solid support suspended in dry N,N-dimethylformamide (5 g support in 22 ml of dimethylformamide). Triethylamine (about 1.0 ml triethylamine per gram of derivatized support), is added, and the mixture shaken briefly. The period of time used for reaction between the p-nitrophenyl ester and the support, as well as the concentration of the p-nitrophenyl ester, can be varied to give different levels of loading the support, as shown below in Table I. The support on which the deoxyribonucleoside has been loaded is then washed, as with N,N-dimethylformamide (five times), methanol (five times), and ether (twice), and then dried under vacuum.

TABLE I

LOADING OF SOLID METHACRYLATE-VINYLIDENE POLYMERIC SUPPORT WITH ACTIVATED PROTECTED THYMIDINE

Concentration of Activated Protected Thymidine, mole/l	Reaction Time, min.	Nucleoside Loading, μmole/g
0.2	180	70-90
0.1	30	40-50
0.05	30	25-35
0.01	30	10-15

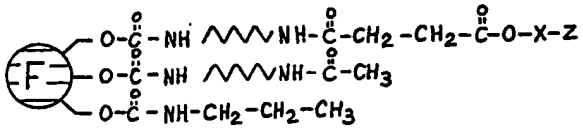
Table I shows that the quantity of nucleoside loaded per gram of support varies with the time of reaction and the concentration of activated protected nucleoside used. The longer the time or the greater the concentration, the greater is the quantity of nucleoside loading that results.

3. The Capping Step

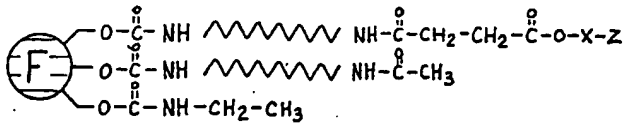
Preferably, the final step of the reaction is the capping of all remaining unreacted amino groups on the nucleoside-loaded solid support. Preferably, the capping is accomplished by acylation. Most preferably, the capping is performed by acetylation.

For capping by acetylation, the support is resuspended in dry pyridine (1 g support per 10 ml pyridine). N,N-dimethylaminopyridine is added to 0.2 mole/l, followed by acetic anhydride to 2.5 mole/l. The reaction mixture is shaken overnight at a temperature of 15°-30°C, preferably room temperature. The solid support is filtered and washed extensively, as with N,N-dimethylformamide (five times), acetonitrile (five times), dichloromethane (five times), and then once with ether. The derivatized, capped solid support is then dried under vacuum. The derivatized support is stable and can be stored.

The result of this process is a derivatized support, four examples of which are shown below as schematic formulas I-IV.



(I)



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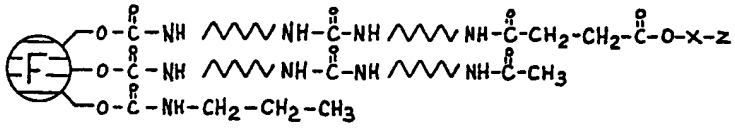
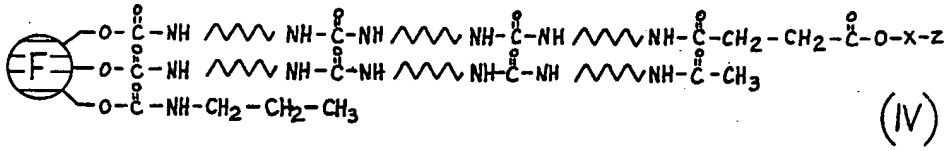


(III)



(IV)

In these formulas, Ⓕ represents the methacrylate-vinylidene porous solid support, X represents a nucleoside, and Z represents a 5'-protecting group for the nucleoside. The diagonal lines, by convention, represent methylene ($-CH_2-$) groups. These formulas show the blocking of unreacted hydroxy groups on the porous solid support by reaction with an amine, and the capping of remaining free amino groups (either from the blocking reaction or from the linker) by acetylation.

The support can be prepared with any DNA or RNA base attached to the support: adenine, thymine, guanine, or

cytosine for DNA, and uracil instead of thymine in the case of RNA.

The quantity of protected nucleoside derivatized per gram of solid support ranges from about 5 μmole/g to about 200 μmole/g. Typically, the quantity is about 30 μmole/g. As shown above in Table I, the level of loading of nucleoside can be varied by varying the time of reaction of the p-nitrophenyl ester with the support and varying the concentration of p-nitrophenyl ester used for reaction.

The fully derivatized support is thoroughly washed and filtered, then dried. Several different washing regimes are possible. For support derivatized by one alkanediamine, the support can be washed five times with dichloromethane, ten times with acetone, five times with acetonitrile, and five times again with dichloromethane. The last dichloromethane filtrate is then tested qualitatively for amino groups using the ninhydrin test, in which a positive result (i.e., the presence of amino groups) yields a blue color and a negative result yields no color. The ninhydrin test should be negative. The support is then washed twice with ether and dried under vacuum, preferably about five hours. For a support derivatized with two alkanediamine moieties, the solid support can be washed with dichloromethane and then 95% ethanol. The filtrates can be collected and assayed by absorbance at 402 nanometers (A_{402}) for determining p-nitrophenol, which is a measure of the quantity of amino groups attached to the support. Other washing schemes are possible, as long as they substantially remove unreacted reagents.

B. Support with Polyethylene Glycol Linker

Another version of the process results in a support incorporating a polyethylene glycol linker.

In general, this process preferably comprises the steps of:

(1) coupling:

(a) a porous solid-phase support comprising a methacrylate-vinylidene copolymer extended with polyethylene glycol, the polyethylene glycol having from about 2 to about 20 ethylene glycol moieties and containing a terminal amino group, with:

(b) a nucleoside covalently linked to the terminal amino group through an amide linkage; and

(2) acylating terminal amino groups that are not coupled to nucleosides.

Preferably, the nucleoside is a protected deoxyribonucleoside.

This results in the nucleoside being linked to the terminal amino group of the polyethylene glycol-derivatized polymer. Accordingly, the nucleotide synthesis intermediate produced thereby comprises:

(1) a porous solid-phase particulate support comprising a methacrylate-vinylidene copolymer;

(2) a polyethylene glycol linker having from about 2 to about 20 ethylene glycol moieties and having a first end and a second end, the first end covalently attached to the particulate support, the second end terminating in an amine moiety; and

(3) a nucleoside linked to the amine moiety at the second end of the polyethylene glycol linker by an amide

linkage between the amine moiety and a carboxyl group in covalent linkage with the nucleoside.

The length of the polyethylene glycol linker is preferably from about 3 to about 10 monomers, most preferably from about 4 to about 7 monomers.

Typically, the particles of the porous particulate support are from about 10 microns to about 200 microns in mean diameter; preferably from about 30 microns to about 70 microns; most preferably, they are about 60 microns in mean diameter.

1. The Coupling Reaction

The coupling reaction between the activated aryl ester of the protected deoxyribonucleoside and the solid support occurs in two stages, as described above in Section II(A)(2).

In a preferred embodiment, in which the activated aryl ester is a p-nitrophenyl ester and the deoxyribonucleoside is succinylated, the formation of the p-nitrophenyl ester of the succinylated protected deoxyribonucleoside is described in Section II(A)(2), supra.

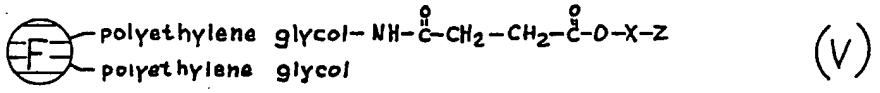
The p-nitrophenyl ester is reacted with the amino-derivatized support by the following procedure: The support is suspended in dry N,N-dimethylformamide. The p-nitrophenyl ester is added as a supernatant from the coupling procedure, and triethylamine is added (1 ml per 2 ml dimethylformamide). The reaction mixture is shaken at a temperature between about 15°C and about 30°C for about 15

minutes to about one hour. Preferably, the reaction is performed at room temperature for about 30 minutes.

The solid support on which the deoxyribonucleoside has been loaded is then filtered and successively washed with N,N-dimethylformamide (three times), methanol (two times), and ether (two times), and dried under vacuum.

2. The Acetylation Reaction (Capping Step)

The acetylation reaction is performed as detailed in Section II(A)(2), supra, in dry pyridine, with the addition of N,N-dimethylaminopyridine and acetic anhydride. The acetylated support is washed successively with N,N-dimethylformamide, acetonitrile, and dichloromethane, and dried under vacuum. The derivatized solid support produced by this method has the schematic structure shown below as Formula V.



(V)

C. Support Derivatized with Epichlorohydrin

Another version of the process first reacts the support with epichlorohydrin (1-chloro-2,3 epoxypropane), and then reacts the epoxy function of the epichlorohydrin with an alkanediamine.

In general, this version of the process comprises the steps of:

(1) condensing a porous solid-phase particulate support comprising a porous polymer as described above, with epichlorohydrin;

(2) condensing:

(a) the reaction product between the polymer and epichlorohydrin with:

(b) at least one aliphatic diamine as described above, to form a modified solid phase support containing at least one diamine linked to the solid support by a spacer with the structure $-O-CH_2-CHOH-CH_2-$, the modified solid phase support having a terminal amino group available for reaction with a nucleoside;

(3) reacting the modified solid phase support with a short-chain alkylamine;

(4) coupling the terminal amino group of the solid phase support with a nucleoside; and

(5) acetylating terminal amino groups remaining uncoupled.

Preferably, the nucleoside is a protected deoxyribonucleoside

The nucleotide synthesis intermediate thus produced comprises:

(1) a porous solid-phase particulate support comprising a porous organic polymer, at least partially hydrophilic and having hydroxy residues, whose backbone comprises straight-chain optionally substituted acrylate or methacrylate moieties;

(b) a linear polymer having first and second ends, the linear polymer being covalently linked through the first end to the particulate support, the linear polymer comprising at least one aliphatic diamine as described above, the aliphatic diamines having a total chain length of from about 6 to about 36 carbon atoms, the linkage between the particulate

support and the first end of the linear polymer comprising a spacer with the structure $-CH_2-CH(OCOCH_3)-CH_2-$, with the first amino group of the diamine moiety attached to the particulate support being substituted with an acetyl group; and

(3) a nucleoside linked to the second end of the linear polymer.

The linear polymer that links the particulate support and the nucleoside has an amino group at its second end available for reaction with the nucleoside. If more than one diamine is present in the linear polymer, the diamines are linked to each other by carbonyl groups.

Preferably, only one diamine is used. Most preferably, the single diamine has a chain length of twelve carbon atoms.

1. The Reaction With Epichlorohydrin

(a) Preferred Polymers

Preferred polymers in this version are the same as those described above (Section II(A)(I)(a), supra). A methacrylate-vinylidene copolymer that is particularly preferred has a mean particle diameter of about 45 microns. This polymer has pores of a size that exclude proteins of molecular weight greater than about $5x10^6$.

(b) Reaction Conditions

The hydroxy-terminated polymer is slightly moistened with a small amount of water and suspended in a 1.5 N-2.5 N solution of alkali metal hydroxide. Preferably the concentration of alkali metal hydroxide is about 2 N and the

alkali metal hydroxide is sodium hydroxide. Epichlorohydrin is added to a final concentration of from about 2.4 mole/l to about 2.7 mole/l; preferably, the concentration of epichlorohydrin is about 2.56 mole/l. The mixture is shaken for about 2-4 hours, preferably about 3 hours. The epichlorohydrin-derivatized solid support is filtered and washed with distilled water until the washes have a neutral pH.

2. Condensation Reaction

The epichlorohydrin-derivatized polymer is subsequently condensed with an alkanediamine moiety. The epichlorohydrin-derivatized polymer is resuspended in N,N-dimethylformamide, and the alkanediamine is added to a concentration of about 0.5 mole/l. The mixture is shaken overnight at a temperature from about 15°-30°C, preferably room temperature. If desired, additional alkanediamine moieties can be added as described above.

3. The Blocking Step

All unreacted epichlorohydrin moieties remaining on the solid support are then blocked by reaction with a short-chain aliphatic primary amine. Preferably, the primary amine is n-propylamine. The blocking step is carried out as follows: The short-chain aliphatic primary amine is added to a concentration of about 1 mole/l and the reaction mixture is shaken for an additional period of about two hours.

Subsequently, the solid support is filtered and washed successively with, e.g., dimethylformamide (five times), acetone (five times), acetonitrile (five times),

dichloromethane (five times) and ether (two times). The support is then dried under vacuum.

4. The Coupling Step

The coupling step is carried out as described above in Section A. The quantity of nucleoside loading can be varied by varying the concentration of activated nucleoside and the time of reaction, as shown in Table II.

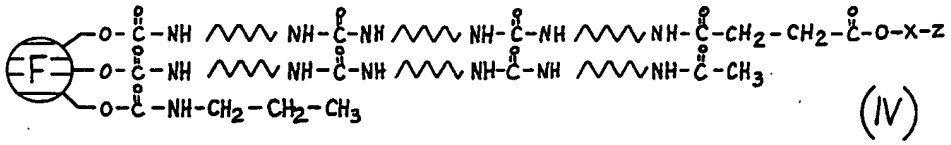
TABLE II

LOADING OF SOLID SUPPORT DERIVATIZED WITH EPICHLOROHYDRIN WITH ACTIVATED PROTECTED THYMIDINE

Concentration of Activated Protected Thymidine, mole/l	Reaction Time min.	Nucleoside Loading, μmole/g
0.2	120	90-120
0.1	30	50- 60
0.05	30	40- 50
0.02	30	25- 35
0.005	30	10- 15

5. The Capping Step

The final step of the process is a capping step, in which all remaining unreacted amino groups and hydroxyl groups are capped by acetylation. This is carried out as described above in Section II(A)(2), supra. After acetylation, the support is filtered and washed successively with N,N-dimethylformamide, acetonitrile, dichloroethane (five times each), then twice with ether, and dried under vacuum for about five hours. The resulting derivatized support has the structure shown below in Formula VI. In Formula VI, Ac represents acetyl (-$COCH_3$).



(IV)

III. USE OF THE SOLID SUPPORTS IN OLIGODEOXYRIBONUCLEOTIDE SYNTHESIS

The solid-phase supports of the present invention are particularly suitable for use with either the phosphite-triester or the phosphotriester method of oligonucleotide synthesis.

In general, and with respect to the 3' to 5' synthesis of deoxyribonucleotides on a solid support, the phosphite-triester method comprises the steps of:

(1) selecting a suitable solid-phase nucleotide synthesis intermediate prepared as described above, the nucleotide synthesis intermediate containing a 5'-protected deoxyribonucleoside;

(2) removing the protecting group of the 5'-protected deoxyribonucleoside attached to the solid support by treating the intermediate with acid to yield a free 5'-OH group;

(3) coupling the free 5'-OH group with a deoxyribonucleoside 3'-phosphoramidite having a 5'-protected hydroxyl to form a phosphite linkage between the 5'-carbon of the deoxyribonucleoside attached to the solid support and the deoxyribonucleoside residue of the phosphoramidite;

(4) oxidizing the phosphite triester linkage to a phosphotriester linkage to yield a dinucleotide attached to the solid support;

(5) acetylating remaining unreacted 5'-OH groups after the coupling steps;

(6) repeating, for each additional nucleotide residue added to the chain, the steps of removing the protecting group, coupling the resulting free 5'-OH group with a deoxyribonucleoside 3'-phosphoramidite having a 5'-protected hydroxyl to form a phosphite linkage, oxidizing the phosphite linkage to a phosphotriester linkage, and acetylating remaining unreacted 5'-OH groups; and

(7) cleaving the oligodeoxyribonucleotide from the solid support.

In general, and with respect to the 3' to 5' synthesis of deoxyribonucleotides on a solid support, the phosphotriester method comprises the steps of:

(1) selecting a suitable solid-phase nucleotide synthesis intermediate prepared as described above, the nucleotide synthesis intermediate containing a 5'-protected deoxyribonucleoside;

(2) removing the protecting group of the 5'-protected deoxyribonucleoside attached to the solid support by

treating the intermediate with acid to yield a free 5'-OH group;

(3) coupling the free 5'-OH group with a 3'-O-phosphorylated deoxyribonucleoside having a protected 5'-OH group in the presence of a coupling agent and catalyst to form a dinucleotide attached to the solid support;

(4) optionally, acetylating remaining unreacted 5'-OH groups after the coupling steps;

(5) repeating, for each additional nucleotide residue added to the chain, the steps of removing the protecting group, coupling the free 5'-OH group with a 3'-O-phosphorylated deoxyribonucleoside, and optionally, acetylating 5'-OH groups remaining unreacted; and

(6) cleaving the completed oligodeoxyribonucleotide from the solid support.

In this procedure, the coupling agent is preferably 1-mesitylenesulfonyl-3-nitro-1,2,4-triazole and the catalyst 1-methylimidazole.

The phosphite-triester and phosphotriester procedures are well known in the art and are described in Gait, supra.

Solid supports according to the present invention can be used for synthesis in automated equipment, including, but not limited to, the Gene Assembler™ (Pharmacia, Uppsala, Sweden), the Biosearch™ 8750 (Milligen Biosearch, San Rafael, California), and the ABI PCR Mate™ (ABI, Foster City ,California). The use of automated equipment is well known in the art of oligodeoxyribonucleotide synthesis. Solid supports, according to the present invention, can be used to synthesize oligodeoxyribonucleotides up to at least 1000 residues in length.

The following Examples are for illustrative purposes only and are not to be construed as limiting the scope of the invention, or the claims to follow, in any manner.

EXAMPLES

Example 1

Synthesis of Macroreticular Methacrylate-Vinylidene Copolymer Derivatized With Diaminododecane Spacer Coupled to Thymidine

FRACTOGEL™-65F (Tosohaas, Philadelphia, Pennsylvania) (10 g, dry weight) was suspended in 100 ml of dry acetonitrile. The condensing agent 1,1'-carbonyldiimidazole (16.2 g, to make a solution of 1 mole/l), was added and the reaction mixture was shaken at room temperature for four hours. The solid support was filtered on a sintered glass funnel and washed four times with 100 ml of dry acetonitrile each time. The solid support was then resuspended in 100 ml of dry dichloromethane.

The spacer, 1,12-diaminododecane (20 g, to make a solution of 1 mole/l) was added and the reaction mixture was shaken at room temperature overnight (i.e., for 16-20 hours). The blocking agent n-propylamine (8.2 ml, to make a solution of 1 mole/l) was added to the above mixture. The mixture was then shaken for an additional hour.

The support was filtered and washed successively five times with 100 ml each time of dichloromethane, ten times with 100 ml each time of acetone, five times with 100 ml each time of acetonitrile, and five times with 100 ml each time of dichloromethane. After the last of the dichloromethane

washes, the filtrate was tested qualitatively by reaction with ninhydrin. The support was then washed twice with 100 ml each time of ether. The support was dried under vacuum for about five hours.

A qualitative ninhydrin test was performed for monitoring amino groups on the solid support. For the ninhydrin test, Reagent 1 was 0.01 mole/l aqueous KCN (2 ml) diluted to 100 ml with pyridine. Reagent 2 was a 5% ninhydrin solution in n-butanol. Reagent 3 was 80% phenol in n-butanol. An aliquot (about 3-5 mg) of the solid support was placed in a test tube and equal amounts (about 5 drops each) of Reagents 1, 2, and 3 were successively added. The test tube was placed in a preheated block at 100°C for 5 minutes. The beads gave a dark blue color, which indicated a positive amine reaction.

Additionally, the amino groups on the solid support were quantitated by a quantitative picric acid test. The reagents used were Reagent A, 5% (v/v) diisopropylethylamine in dichloromethane; and Reagent B, 0.1 mole/l picric acid in dichloromethane. An accurately weighed amount of the solid support (5-10 mg) was placed in a small column fitted with a Teflon™ stopcock. The solid support was washed twice with 2 ml each of dichloromethane and then treated twice with 2 ml of Reagent A for one minute each. The support was then washed five times with 1 ml each of dichloromethane for one minute each time. The solid support was then treated twice with Reagent B, 2 ml each time, for one minute each time. The support was washed five times with dichloromethane, 1 ml each time, for one minute each time. The picrate was eluted twice with Reagent A (2 ml each time, 1 minute each). The picrate was diluted with 95% ethanol to give an absorbance measurable at 358 nm in the spectrophotometer. The amino group content

on the solid support was calculated using the following formula:

$$\text{Amino groups } (\mu\text{mole/g}) = \frac{A_{358} \times V \times 1000}{14.5 \times W}$$

In this formula, A_{358} is the measured absorbance at 358 nm, V is the volume of the solution in milliliters, E x 10^{-3} is 14.5, and W is the weight of the sample used in the test in milligrams.

The amino group content on the solid support was typically 300-400 μmole/g.

The next step in the preparation of the derivatized solid support was loading the amino-containing solid support with 5'-dimethoxytrityl-thymidine. Different concentrations of the 5'-dimethoxytrityl-thymidine succinates were prepared according to the required nucleoside loading. The particular procedure set forth here was used for preparing a 0.05 mole/l solution to load 5 g of the solid support.

The succinylated 5'-dimethoxytrityl-thymidine (2.5 mmole) was dissolved in 22 ml of dry dioxane containing 1 ml of dry pyridine and 350 mg of p-nitrophenol (25 nmole). The condensing agent (1,3-dicyclohexylcarbodiimide; 1.29 g, 6.25 mmole) was then added. After a few minutes dicyclohexylurea began to precipitate. The reaction mixture was shaken at room temperature for three hours. The dicyclohexylurea was then removed by filtration and the supernatant was added to the amino-derivatized solid support (5 g) suspended in dry N,N-dimethylformamide (22 ml).

Triethylamine (5 ml) was added to the above mixture and the mixture was shaken briefly by hand. A bright yellow

color developed, due to the release of p-nitrophenol. The reaction was shaken for a period of time sufficient to give the required nucleoside loading. (See Table I above).

The supernatant was removed and used to derivatize another aliquot (2-3 g) of amino-derivatized solid support because it still contained activated deoxyribonucleoside.

The deoxyribonucleoside-derivatized solid support was washed extensively: 5 times with 100 ml each of N,N-dimethylformamide, 5 times with 100 ml each of methanol, and twice with 100 ml each of ether. The solid support was then dried under vacuum.

The remaining amino groups on the deoxyribonucleoside-loaded solid support were then capped by acetylation. The solid support (5 g) from the previous step was suspended in 50 ml of dry pyridine. N,N-dimethylaminopyridine (1.25 g, to make an 0.2 mole/l solution) was added followed by the addition of acetic anhydride (12.5 ml, to make a solution of 2.5 mole/l). The mixture was shaken at room temperature overnight.

The solid support was filtered and washed successively with 5 x 50 ml each of N,N-dimethylformamide, acetonitrile, and dichloromethane, and once with ether. The support was then dried under vacuum for about 5 hours. The amine-capped solid support was tested by the ninhydrin test for complete capping. The test showed that there were no free (i.e., uncapped) amino groups remaining on the solid support.

Example 2

Synthesis of Macroreticular Methacrylate-Vinylidene Copolymer Derivatized With Diaminododecane Spacer Coupled To Deoxycytidine

A solid support derivatized with a diaminododecane spacer coupled to deoxycytidine was prepared as in Example 1, except that the protected deoxyribonucleoside used was benzoyl-dimethoxytrityl-deoxycytidine succinate. This protected and activated deoxycytidine derivative was substituted for dimethoxytrityl-thymidine succinate used in Example 1.

Example 3

Synthesis of Macroreticular Methacrylate-Vinylidene Copolymer Derivatized With Diaminododecane Spacer Coupled To Deoxyguanosine

A solid support derivatized with a diaminododecane spacer coupled to deoxyguanosine was prepared as in Example 1, except that isobutyryl-dimethoxytrityl-deoxyguanosine succinate was substituted for dimethoxytrityl-thymidine succinate, as used in Example 1.

Example 4

Synthesis of Macroreticular Methacrylate-Vinylidene Copolymer Derivatized With Diaminododecane Spacer Coupled To Deoxyadenosine

A solid support derivatized with a diaminododecane spacer coupled to deoxyadenosine was prepared as in Example 1, except that benzoyl-dimethoxytrityl-deoxyadenosine succinate was substituted for dimethoxytrityl-thymidine succinate, as used in Example 1.

The nucleoside loading on the solid supports for Examples 1-4 was determined using two methods. The first method was quantitating the released dimethoxytrityl color. The second was by quantitating the released nucleoside. The released nucleoside was quantitated by determining its ultraviolet absorption.

For the first method, an accurately weighed amount (428 mg) of the solid support was placed in a disposable column of 5 ml capacity. A solution of dichloroacetic acid (2.5% in dichloromethane) was added to the solid support (about 1 ml) and shaken for one minute, and then filtered into a measuring cylinder. Another 1-ml portion was added, shaken for one minute, and filtered into the same cylinder. The volume was brought to 10 ml with the same dichloroacetic acid solution. The optical density was measured at 500 nm using the dichloroacetic acid solution as a blank. The nucleoside loading was estimated from the DMT assay using the following formula:

$$DMT = \frac{A_{500} \times V \times 1000}{80 \times W}$$

where A_{500} is the measured absorbance at 500 nanometers, V is the volume in milliliters of dichloroacetic acid solution used, 80 is the molar absorbance times $1x10^{-3}$ of DMT at 500 nm, and W is the weight in mg of the solid support.

For the second method, nucleoside release, the solid support from step two of the first method was washed with 5 ml of dichloromethane and 5 ml of acetonitrile, and then dried. The solid support was treated with 1 ml of ammonia at room temperature for one hour to bring about hydrolysis of the 3'-succinate bond. The ammonia solution was transferred to a screw-cap vial, capped tightly, and sealed well with parafilm.

The vial containing the nucleoside was placed in a water bath at 65°C for three hours. The vial was cooled before opening, left open at room temperature for at least 30 minutes, and then evaporated to dryness using a vacuum concentrator. The residue was dissolved in 1 ml of double distilled water. The ultraviolet absorbance was read at the following wavelengths: 264 nm for thymidine; 260 nm for deoxyadenosine; 269 nm for deoxycytidine; and 255 nm for deoxyguanosine. Nucleoside loading was determined according to the following formula:

$$\text{Nucleoside loading} = \frac{A \times 1000}{E \times W}$$

where E is the extinction coefficient for the particular deoxyribonucleoside in μmole/ml; 15.26 for deoxyadenosine; 7.70 for deoxycytidine; 11.50 for deoxyguanosine; and 8.83 for thymidine), and W is the weight of the solid support in mg.

The values obtained for loading by the two methods are shown below in Table III. As can be seen, there is substantial agreement between the two methods.

TABLE III

NUCLEOSIDE LOADING FOR EXAMPLES 1-4

Nucleoside Loaded	Loading Determined by DMT Release, μmole/g	Loading Determined by Nucleoside Release, μmole/g
T (Ex. 1)	29.96	28.10
A (Ex. 2)	34.70	34.20
C (Ex. 3)	36.50	34.41
G (Ex. 4)	35.90	35.90

Example 5

Synthesis of Macroreticular Methacrylate-Vinylidene Copolymer Derivatized With Polyethylene Glycol Linker Coupled to Thymidine

To prepare a solid support derivatized with a polyethylene glycol linker coupled to thymidine, FRACTOGEL™-65M (AF-amino-650M, Tosohaas, Philadelphia, Pennsylvania) was used. This macroreticular methacrylate-vinylidene copolymer has amino-terminated polyethylene glycol residues attached thereto.

Succinylated dimethoxytrityl-thymidine (3.23 mg, 0.5 mmole) was dissolved in 4 ml of dry dioxane containing 0.2 ml of dry pyridine and 70 mg of p-nitrophenol (0.5 mmole). The coupling agent 1,3-dicyclohexylcarbodiimide (259 mg, 1.25 mmol) was then added. After a few minutes dicyclohexylurea began to precipitate. The reaction mixture was shaken at room temperature for three hours. The dicyclohexylurea was removed by filtration and the supernatant was added to the amino-derivatized methacrylate-vinylidene copolymer (1 g, amino groups = 400 μmole/g) suspended in 4 ml of dry N,N-dimethylformamide.

Triethylamine (2 ml) was added to the above reaction mixture. The reaction mixture was shaken at room temperature for 30 minutes.

The solid support was filtered and successively washed with N,N-dimethylformamide (3 times with 10 ml each), methanol (twice with 10 ml each), and ether (twice with 10 ml each), and dried under vacuum for about five hours. In order to cap the remaining amino groups on the nucleoside-loaded solid support by acetylation, the solid support from the

previous step (1 g) was suspended in 10 ml of dry pyridine. N,N-dimethylaminopyridine (250 mg, to make a solution of 0.2 mole/l) was added followed by 2.5 ml of acetic anhydride (to make a 2.5 mole/l solution). The reaction mixture was shaken at room temperature overnight. The solid support was filtered and washed successively with N,N-dimethylformamide, acetonitrile, and dichloromethane (5 times with 10 ml of each solvent), and then twice with ether (10 ml each time) and dried under vacuum for about five hours.

The solid support was tested for complete capping by the ninhydrin test. A negative result was obtained, (i.e., no blue color), indicating that there were no free amino groups remaining. The nucleoside loading on the solid support was estimated by quantitating the released DMT color as described in Example 1. The nucleoside loading was determined to be 28 μmole/g.

Example 6

Synthesis of Macroreticular Methacrylate-Vinylidene Copolymer Derivatized With Two Diaminohexane Spacers Joined By Carbonyl Linkage and Coupled To Thymidine

A procedure similar to that disclosed in Example 5 was used to couple two diaminohexane residues to an hydroxyl-terminated macroreticular methacrylate-vinylidene copolymer.

FRACTOGEL™-65F (10 g dry weight) was suspended in 100 ml of dry acetonitrile. The coupling agent, 1,1'-carbonyldiimidazole (16 g, to make a solution of 1 mole/l) was added and the reaction mixture was shaken at room temperature for four hours. The solid support was filtered on a sintered glass funnel and washed four times with 100 ml each time of dry acetonitrile.

The washed solid support was then resuspended in 100 ml of dry acetonitrile. The first diaminohexane spacer was coupled by adding 11.6 g of 1,6-diaminohexane to make a 1 mole/l solution. The mixture was shaken at room temperature overnight. Next, 8.2 ml n-propylamine (to make 1 mole/l) was added. The mixture was then shaken for an additional hour. The solid support was then filtered and washed successively ten times with 100 ml each time of acetonitrile, twenty times with 100 ml each time of distilled water, three times with 100 ml each time of acetonitrile, three times with 100 ml each time of dichloromethane, and twice with 100 ml each time of ether. The solid support was then dried under vacuum for about five hours. A picric acid assay showed an amino group loading of about 500-600 μmole/g.

To add a second diaminohexane moiety to the solid support, 10 g of the solid support was suspended in 100 ml of dry acetonitrile. The coupling agent 1,1'-carbonyldiimidazole (16.2 g to make a solution of 1 mole/l) was added, and the mixture was shaken at room temperature for four hours. The solid support was then filtered and washed five times with 100 ml each of dry acetonitrile and resuspended in 100 ml of dry acetonitrile. The second diamine moiety, 1,6-diaminohexane, was added (11.6 g to make a solution of 1 mole/l). The mixture was then shaken at room temperature overnight. The solid support was then filtered and washed successively ten times with 100 ml each of acetonitrile, twenty times with 100 ml each of distilled water, three times with 100 ml each of acetonitrile, three times with 100 ml each of dichloromethane, and twice with 100 ml each with ether. The solid support was then dried under a vacuum for about five hours. The picric acid assay showed that the amino group loading was about 350-400 μmole/g.

The next step was the loading of the amino-terminated solid support with thymidine. Succinylated 5'-dimethoxytrityl-thymidine (1.61 g, 2.5 mmole) was dissolved in 22 ml of dry dioxane containing 1 ml of dry pyridine and 330 mg of p-nitrophenol (2.5 mmole). The condensing agent, 1,3-dicyclohexylcarbodiimide (1.29 g, 6.25 mmole) was then added. After a few minutes, dicyclohexylurea began to precipitate. The reaction mixture was shaken at room temperature for 3 hours. The dicyclohexylurea was removed by filtration and the supernatant was added to the amino-derivatized solid support (5 g) suspended in 22 ml of dry N,N-dimethylformamide. Triethylamine (5 ml) was added to the above reaction mixture, which was then shaken briefly by hand. A bright yellow color developed due to the release of p-nitrophenol. The reaction mixture was then shaken at room temperature for thirty minutes. The solid support was then filtered and successively washed five times with 100 ml each time of N,N-dimethylformamide, five times with 100 ml each time of methanol, and twice with 100 ml each of ether, and then dried under vacuum for about five hours.

The next step was the capping of the remaining amino groups on the nucleoside-loaded solid support by acetylation. The solid support from the previous step (5 g) was suspended in 50 ml of dry pyridine. N,N-dimethylaminopyridine (1.25 g, to make a 0.2 mole/l solution) was added followed by 12.5 ml of acetic anhydride (to make a 2.5 mole/l solution). The reaction mixture was shaken overnight at room temperature. The solid support was filtered and washed successively five times with 50 ml each time of N,N-dimethylformamide, acetonitrile, and dichloromethane, and then with 250 ml of ether. The support was then dried under a vacuum for about 5 hours. The amino-capped solid support was tested by the ninhydrin test for complete capping, which gave a negative

result. The nucleoside loading on the solid support was estimated by quantitating the released dimethoxytrityl color; the nucleoside loading was determined to be 39 μmole/g.

Example 7

Synthesis of Macroreticular Methacrylate-Vinylidene Copolymer Derivatized With Two Diaminohexane Spacers Joined By Carbonyl Groups and Coupled To Thymidine, With Second Diaminohexane Spacer Coupled by Reaction With *p*-Nitrophenylchloroformate

The solid support, FRACTOGEL™-75F (exclusion limit $5x10^7$ molecular weight for proteins; particle size 30-60 microns) (1 g dry weight) was suspended in 20 ml of dry acetonitrile. The coupling agent 1,1'-carbonyldiimidazole (3.24 g, to make a solution of 1 mole/l) was added and the mixture was shaken at room temperature for four hours. The solid support was filtered on a sintered glass funnel and washed five times with dry acetonitrile (10 ml each time).

The solid support was then resuspended in 20 ml of dry acetonitrile. The first diamine moiety, 1,6-diaminohexane (2.32 g, to make a solution of 1 mole/l) was added and the mixture shaken at room temperature overnight. The blocking agent n-propylamine (1.64 ml) was added to the above mixture. The mixture was then shaken for an additional hour. The solid support was filtered and washed successively ten times with 10 ml of acetonitrile each time, twenty times with 10 ml of distilled water each time, three times with 10 ml of acetonitrile each time, three times with 10 ml of dichloromethane each time, and twice with 10 ml of ether each time. The solid support was then dried under a vacuum for about five hours. The picrate assay showed that amino group loading was about 250-270 μmole/g.

To add a second diaminohexane moiety, the solid support (1 g) was suspended in 20 ml of dry dichloromethane. The coupling agent p-nitrophenylchloroformate (4.02 g, to make a solution of 1 mole/l) was added, followed by addition of 3.95 ml collidine (to make a 1.5 mole/l solution). The mixture was then shaken at room temperature overnight.

The solid support was then filtered and washed five times with dry dichloromethane (20 ml each time) and resuspended in 20 ml of dry dichloromethane. The second diamine moiety, 1,6-diaminohexane (2.32 g, to make a solution of 1 mole/l) was added, followed by 3.03 ml triethylamine, to make a 1.5 mole/l solution, and the mixture shaken at room temperature overnight. The solid support was filtered and washed twice with 10 ml each time of dichloromethane and five times with 10 ml each time of 95% ethanol. The filtrates were collected and brought up to 50 ml with 95% ethanol to be used for the p-nitrophenol assay, as described below. The solid support was further washed five times with methanol (10 ml each time), five times with acetonitrile (10 ml each time), five times with dichloromethane (10 ml each time), and twice with 10 ml each time of ether. The support was dried under vacuum for about five hours.

For the p-nitrophenol assay, the p-nitrophenol-diaminohexane salt solution obtained from the above reaction was appropriately diluted and read for absorbance at 402 nm. The amount of p-nitrophenol released in the reaction, which corresponds to the amount of amino groups attached, was calculated from the formula:

$$\text{Nitrophenol release } (\mu\text{mole/g}) = \frac{A_{402} \times V \times 1000}{21 \times W}$$

where A_{402} is the absorbance at 402 nm, V is the final volume in milliliters of the solution used to measure the absorbance, 21 is the extinction coefficient x 10^{-3} of the p-nitrophenol amine complex at 402 nm, and W is the weight of the solid support in grams.

The assay indicated that the amount of p-nitrophenol released was 200 to 240 μmole/g. By the picrate acid assay, the amino group loading was 190 to 240 μmole/g.

The next step was the loading of the amino-terminated solid support with thymidine. Succinylated 5'-dimethoxytrityl-thymidine (1.29 g, 2 mmole) was dissolved in 4 ml of dry dioxane containing 0.4 ml of dry pyridine and 280 mg of p-nitrophenol (2 mmole). The coupling agent 1,3-dicyclohexylcarbodiimide (1.30 g, 5 mmole) was then added. After a few minutes, dicyclohexylurea began to precipitate. The reaction mixture was shaken at room temperature for 3 hours.

The dicyclohexylurea was removed by filtration and the supernatant was added to the amino-derivatized solid support (1 g) suspended in 4 ml of dry N,N-dimethylformamide. Triethylamine (2 ml) was added to the above reaction mixture. The reaction mixture was then shaken at room temperature overnight.

The solid support was then filtered and successively washed 3 times with 10 ml each time of N,N-dimethylformamide, twice with 10 ml each time of methanol, and twice with 10 ml each time of ether. The solid support was then dried under vacuum for about 5 hours.

The final step was the capping of the remaining amino groups on the nucleoside-loaded solid support by acetylation. The solid support (1 g) from the previous step was suspended in 10 ml of dry pyridine. N,N-dimethylaminopyridine (250 mg, to make a solution of 0.2 mole/l) was added, followed by the addition of 2.5 ml acetic anhydride (to make a solution of 2.5 mole/l). The reaction mixture was shaken at room temperature overnight. The solid support was then filtered and washed successively 5 times with 10 ml each time with the following reagents: N,N-dimethylformamide, acetonitrile, and dichloromethane. The solid support was then washed twice with 10 ml each time of ether and then dried under vacuum for about 5 hours.

The nucleoside loading on the solid support was 28 to 30 μmole/g, estimated by quantitating the released DMT color.

Example 8

Synthesis of Macroreticular Methacrylate-Vinylidene Copolymer Derivatized with Epichlorohydrin and Diaminododecane and Coupled to Thymidine

The solid support, FRACTOGEL™-65F (10 g), slightly moistened with a small amount of water (about 5 ml), was suspended in 2 N aqueous sodium hydroxide solution (50 ml) by shaking. Epichlorohydrin (10 ml) to make a solution of 2.56 mole/l) was added and the mixture was shaken at room temperature for 3 hours.

The solid support was filtered and washed with distilled water in a sintered glass funnel until the washes were neutral to pH paper. The solid support (slightly wet) was resuspended in 100 ml of N,N-dimethylformamide.

The diamine spacer, powdered 1,12-diaminododecane (10 g, to make a solution of 0.5 mole/l) was added and the mixture was shaken at room temperature overnight (i.e., about 16 to 20 hours). The blocking agent n-propylamine (8.2 ml, to make a solution of 1 mole/l) was added to the above mixture. The mixture was then shaken for an additional 2 hours.

The solid support was then filtered and washed successively 5 times with 100 ml each time of N,N-dimethylformamide, 5 times with 100 ml each time of acetone, 5 times with 100 ml each time of acetonitrile, and 5 times with 100 ml each time of dichloromethane. The last washing with dichloromethane gave a negative ninhydrin test. Finally, the solid support was washed twice with 100 ml each time of ether and dried under vacuum for about 5 hours. The picrate acid assay showed that the amino group loading was about 700 to 800 μmole/g.

The next step was the loading of the amino-terminated solid support with thymidine. Different concentrations of the activated thymidine succinate could be used, according to the required nucleoside loading. In this example, a 0.05 mole/l reagent was prepared to load 1 g of the solid support.

For this procedure, succinylated 5'-dimethoxytrityl-thymidine (323 mg, 0.5 mmole) was dissolved in 4 ml of dry dioxane containing 0.2 ml dry pyridine and p-nitrophenol (70 mg, 0.5 mmole). The coupling agent 1,3-dicyclohexylcarbodiimide (258 mg, 1.25 mmole) was added and the reaction mixture was shaken at room temperature for 3 hours. The dicyclohexylurea was removed by filtration and the supernatant was added to the amino-derivatized solid support (1 g) suspended in 4 ml dry N,N-dimethylformamide. N,N-

triethylamine (2 ml) was added to the reaction mixture. The reaction mixture was shaken for an appropriate time to obtain the required loading of nucleoside.

The loaded solid support was then filtered and successively washed 3 times with 10 ml each time of N,N-dimethylformamide, twice with 10 ml each time of methanol, and twice with 10 ml each time of ether. It was then dried under vacuum for about 5 hours. The nucleoside loading on the solid support was estimated by quantitating the released DMT color.

The last step was the capping of the remaining amino groups on the nucleoside-loaded solid support by acetylation. The solid support (1 g) from the previous step was suspended in 10 ml dry pyridine. N,N-dimethylaminopyridine (250 mg, to make a solution of 0.2 mole/l) was added followed by acetic anhydride (2.5 ml, to make a solution of 2.5 mole/l). The reaction mixture was shaken at room temperature overnight. The solid support was filtered and washed successively with 5 x 10 ml each time of N,N-dimethylformamide, acetonitrile, and dichloromethane, then twice with 10 ml each time of ether. The solid support was dried under vacuum for about 5 hours.

The amino-capped solid support was tested by the ninhydrin test for complete capping. The ninhydrin test gave a negative result, which indicated complete capping of the solid support.

Example 9

DNA Synthesis of 17-Mer Using Derivatized Solid Supports

The following sequence, 5'-TGTAAATGCCATCGACT-3' (SEQ ID NO: 1), was synthesized using the derivatized solid supports described above and several types of commercially available *in vitro* DNA synthesis apparatus.

(a) Synthesis Using Controlled Pore Glass (500 Å) (Control) With Pharmacia Gene Assembler™

As a control, the DNA sequence described was synthesized using controlled pore glass of 500 Å porosity on the Pharmacia Gene Assembler™ DNA synthesis apparatus (Pharmacia, Uppsala, Sweden). The normal procedure for synthesis using this apparatus was followed as provided by the manufacturer. The trityl coupling efficiency for the synthesis is shown below in Table IV.

TABLE IV

TRITYL COUPLING EFFICIENCIES ON PHARMACIA GENE ASSEMBLER

Solid Support of Example No.:	Length of DNA Synthesized:					
	17	24	35	51	70	101
CPG - Thymidine (Control)	99.6	99.3	99.1	98.6	98.5	99.0
1	100.0	99.9	99.1	98.9	98.7	99.1
5	NT	98.6	98.9	99.9	99.3	99.6

Table IV shows that the solid supports of Examples 1 and 5 are at least as efficient as CPG for synthesis of oligonucleotides ranging in length from 17 bases to 101 bases ("NT" means "not tested"). In particular, the solid supports of Examples 1 and 5 proved more efficient than CPG for synthesis of relatively long oligonucleotides, i.e., nucleotides of 51 bases or more. High repetitive yield is most important in synthesizing long oligonucleotides. A brief calculation shows that a repetitive yield of 99% per cycle gives a total yield of 90.4% after 10 cycles and 81.8% after 20 cycles. However, a decrease of only 1% in the repetitive yield, to 98%, gives a total yield of only 81.7% after 10 cycles and 66.8% after 20 cycles.

TABLE V

TRITYL COUPLING EFFICIENCIES ON PHARMACIA GENE ASSEMBLER

Solid Support of Example No.:	Length of DNA Synthesized:
	35
CPG-Thymidine Control	99.1
1	99.1
2	98.6
3	98.9
4	98.8
5	98.9

Table V shows that solid supports according to the present invention can be used to synthesize deoxyribonucleotides efficiently with any of the four common DNA nucleotides attached to the solid support.

(b) Synthesis Using the Solid Support of Example 1 on the Pharmacia Gene Assembler

The solid support of Example 1 was similarly used for synthesis with the Pharmacia Gene Assembler. The trityl coupling efficiency is shown in Table IV above.

It can be seen that the solid support of Example 1 is as efficient in the synthesis of the 17-mer oligodeoxyribonucleotide as is controlled pore glass.

Example 10

DNA Synthesis of 24-Mer Using Derivatized Solid Supports

A 24-nucleotide DNA sequence, 5'-ATTGAGAAAGCGCCACGCTTCCCT-3' (SEQ ID NO: 2), was synthesized using derivatized solid supports as described above and in vitro DNA synthesis apparatus, also as described above.

(a) Synthesis Using Controlled Pore Glass (500 Å) (Control) With Pharmacia Gene Assembler

The 24-mer described above was synthesized using controlled pore glass of 500 Å porosity, as a control, on the Pharmacia Gene Assembler as described above. The trityl coupling efficiency for this synthesis is shown in Table IV.

The DNA synthesized was analyzed by capillary gel electrophoresis. The capillary gel column used was from Beckman Instruments, Fullerton, California (U100P urea gel). The electrophoresis was run on a Beckman P/ACE™ high performance capillary electrophoresis system. A 7 M urea in Tris-hydroxymethylaminomethane-HCl ("Tris-HCl") buffer was

used according to instructions. The capillary gel electropherogram for the synthesized DNA is shown in Figure 1. The results indicate that the synthesized nucleotide was substantially homogeneous.

(b) Synthesis Using Solid Support of Example 1 With Pharmacia Gene Assembler

The same oligonucleotide was synthesized using the solid support of Example 1 on the Pharmacia Gene Assembler DNA synthesis apparatus. The trityl coupling efficiency is shown in Table IV, and the capillary gel electropherogram is shown in Figure 2. The electropherogram showed a single sharp spike. This indicates that the synthesized nucleotide was substantially homogeneous and contained fewer contaminants of differing molecular weight than the nucleotide synthesized with controlled pore glass.

(c) Synthesis Using Solid Support of Example 5 on Pharmacia Gene Assembler

The same oligodeoxyribonucleotide was synthesized using the solid support of Example 5 on the Pharmacia Gene Assembler. The trityl coupling efficiency is shown in Table IV, above, while a capillary gel electropherogram of the synthesized DNA is shown in Figure 3.

(d) Synthesis Using Solid Support of Example 6 on Pharmacia Gene Assembler

The sequence was synthesized using the solid support of Example 6, above, on the Pharmacia Gene Assembler.

(e) Synthesis With Controlled Pore Glass (500 Å Porosity) (Control) On the Biosearch Apparatus

The sequence described above was synthesized using controlled pore glass (500 Å) porosity on the Biosearch™ 8750 DNA synthesis apparatus (Milligen Biosearch, San Rafael, California). The manufacturer's instructions were followed.

Trityl coupling efficiency was determined by measuring spectrophotometrically the released trityl color after the first coupling and the n-1st coupling, and then calculating using the formula:

Stepwise coupling efficiency = $e^{[\ln Y/(n-1)]}$ where Y is (absorbance of color released after n-1 couplings)/(absorbance of color released after the first coupling) and n is the number of nucleotides. The trityl coupling efficiency is shown in Table VI below ("NT" means "not tested").

TABLE VI

TRITYL COUPLING EFFICIENCIES FOR BIOSEARCH 8750

Solid Support of Example No.:	Length of DNA Synthesized				
	24	35	51	70	101
CPG - Thymidine (Control)	98.1	98.3	97.9	NT	NT
1	98.2	98.6	99.1	NT	NT
5	NT	98.8	NT	NT	NT
6	NT	98.5	NT	NT	NT
7	NT	98.7	98.0	98.0	98.4
8	NT	98.8	100.0	100.0	NT

(f) Synthesis Using Solid Support of Example 1 on the Biosearch Apparatus

The same oligodeoxyribonucleotide was also synthesized using the solid support of Example 1 on the Biosearch apparatus. The trityl coupling efficiency is shown in Table VI. The trityl coupling efficiency is substantially equivalent for the solid support of Example 1 and the controlled pore glass control.

(g) Synthesis Using Solid Support of Example 6 on Biosearch Apparatus

The same sequence was also synthesized using the solid support of Example 6 on the Biosearch apparatus. This DNA was used for other studies as described below.

Example 11

DNA Synthesis of 35-Mer Using Derivatized Solid Support

An oligodeoxyribonucleotide of 35 residues, 5'-GATGCCAGTTCGGTCATACACGTAGTACTACGACT-3' (SEQ ID NO: 3), was synthesized using the solid supports described above on various DNA synthesis apparatuses.

(a) Synthesis With Controlled Pore Glass (500 Å Porosity) (Control) on Pharmacia Gene Assembler

Controlled pore glass with 500 Å porosity, as a control, was used to synthesize the sequence on the Pharmacia Gene Assembler. The trityl coupling efficiency is shown in Tables IV and V. A capillary gel electropherogram of the synthesized DNA is shown in Figure 4.

(b) Synthesis Using Solid Support of Example 1 with Pharmacia Gene Assembler

The solid support of Example 1 was used for synthesis with the Pharmacia Gene Assembler. The trityl coupling efficiency is given in Tables IV and V. A capillary gel electropherogram of the synthesized DNA is shown in Figure 5.

(c) Synthesis Using Solid Support of Example 2 with Pharmacia Gene Assembler

The solid support of Example 2 was similarly used for synthesis with the Pharmacia Gene Assembler to synthesize the 35-nucleotide sequence described above. Because the nucleoside attached to the solid support is deoxycytidine for Example 2, the sequence actually synthesized had a C at its 3'-terminal end instead of a T. The trityl coupling efficiency for this synthesis is shown in Table V.

(d) Synthesis Using Solid Support of Example 3 with Pharmacia Gene Assembler

The same 35-residue oligodeoxyribonucleotide was synthesized using the solid support of Example 3 on the Pharmacia gene assembler. Because this solid support has a deoxyguanosine residue attached to it, the nucleotide had a G at its 3'-terminal end rather than a T. The trityl coupling efficiency for this synthesis is shown in Table V.

(e) Synthesis Using Solid Support of Example 4 with Pharmacia Gene Assembler

The solid support of Example 4 was used to synthesize the same 35-residue oligodeoxyribonucleotide. Because the solid support of Example 4 has an deoxyadenosine residue attached to the solid support, the synthesized DNA had an A at its 3'-terminus instead of a T. The trityl coupling efficiency for this synthesis is shown in Table V.

The results of Examples 11(a) through 11(e) indicate that the solid phase support of Example 1 can be used for synthesis with any attached deoxyribonucleoside residue.

(f) Synthesis Using Solid Support of Example 5 with Pharmacia Gene Assembler

The solid support of Example 5 was used for synthesis with the Pharmacia Gene Assembler for synthesis of the 35-residue oligodeoxyribonucleotide whose sequence is given above. (For this and all other portions of Example 11, the solid support has a thymidine residue attached, so the sequence synthesized is identical to the one set out initially at the beginning of the Example.) The trityl coupling efficiency for this synthesis is shown in Tables IV and V. A capillary gel electropherogram of the synthesized DNA is shown in Figure 6.

(g) Use of Solid Support of Example 6 For Synthesis with Pharmacia Gene Assembler

The solid support of Example 6 was used for the synthesis of the 35-residue oligodeoxyribonucleotide given

above with the Pharmacia Gene Assembler. A capillary gel electropherogram of the synthesized DNA is shown in Figure 7.

(h) Use of the Solid Support of Example 8 for Synthesis with the Pharmacia Gene Assembler

The solid support of Example 8 was similarly used with the Pharmacia Gene Assembler for synthesis of the 35-residue oligodeoxyribonucleotide. A capillary gel electropherogram of the synthesized DNA is shown in Figure 8.

(i) Use of Controlled Pore Glass (500 Å Porosity) (Control) for Synthesis with the Biosearch Synthesis Apparatus

Controlled pore glass of 500 Å porosity, a control, was used for synthesis of the 35-residue oligodeoxyribonucleotide described above on the Biosearch 8570 synthesis apparatus. The trityl coupling efficiency is given in Table VI.

(j) Use of Solid Support of Example 1 for Synthesis with Biosearch Synthesis Apparatus

The solid support of Example 1 was used for synthesis with the Biosearch 8750 DNA synthesis apparatus to synthesize the 35-residue oligodeoxyribonucleotide described above. The trityl coupling efficiency is shown in Table V.

(k) Use of Solid Support of Example 5 for Synthesis on Biosearch Synthesis Apparatus

The solid support of Example 5 was used for synthesis of the same 35-residue oligodeoxyribonucleotide on

the Biosearch 8750 synthesis apparatus. The trityl coupling efficiency for this synthesis is shown in Table V.

(l) Use of the Solid Support of Example 6 for Synthesis on the Biosearch Synthesis Apparatus

The solid support of Example 6 was used for synthesis of the same 35-residue oligodeoxyribonucleotide on the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency is shown in Table V.

(m) Use of the Solid Support of Example 7 for Synthesis with the Biosearch Synthesis Apparatus

The solid support of Example 7 was used for synthesis of the same 35-residue oligodeoxyribonucleotide with the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency is given in Table V.

(n) Use of the Solid Support of Example 8 for Synthesis on Biosearch Synthesis Apparatus

The solid support of Example 8 was used for synthesis of the same 35-residue oligodeoxyribonucleotide on the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency is given in Table V.

(o) Use of Controlled Pore Glass (500 Å Porosity) (Control) For Synthesis on ABI Synthesis Apparatus

As a control, controlled pore glass (500 Å porosity) was used for synthesis of the 35-residue oligodeoxyribonucleotide on the ABI PCR Mate DNA synthesis apparatus.

(p) Use of Solid Support of Example 1 for Synthesis with ABI Synthesis Apparatus

The solid support of Example 1 was used for synthesis of the same 35-residue oligodeoxyribonucleotide on the ABI PCR Mate DNA synthesis apparatus.

(q) Use of Solid Support of Example 5 for Synthesis with ABI Synthesis Apparatus

The solid support of Example 5 was used for synthesis of the same 35-residue oligodeoxyribonucleotide with the ABI PCR Mate DNA synthesis apparatus.

Example 12
DNA Synthesis of 51-Mer Using Derivatized Solid Support

A 51-residue oligodeoxyribonucleotide, 5'-TCCATGGCAACTGTCAAGGCACTGGCTCGTAGCCTACTGGCTTGACCGTAT-3' (SEQ ID NO: 4), was synthesized using the solid supports described above in various DNA synthesis apparatus.

(a) Synthesis Of Controlled Pore Glass (500 Å Porosity) (Control) With Pharmacia Synthesis Apparatus

The 51-residue oligodeoxyribonucleotide whose sequence is given above was synthesized with controlled pore glass (500 Å porosity), as a control, with the Pharmacia Gene Assembler DNA synthesis apparatus. The trityl coupling efficiency for this synthesis is shown in Table IV.

(b) Use of Solid Support of Example 1 For Synthesis With Pharmacia Synthesis Apparatus

The solid support of Example 1, above, was used for synthesis of the 51-residue oligodeoxyribonucleotide described above with the Pharmacia Gene Assembler DNA synthesis apparatus. The trityl coupling efficiency for this synthesis is given in Table IV.

(c) Use of Solid Support of Example 5 For Synthesis With Pharmacia Synthesis Apparatus

The solid support of Example 5 was used for synthesis of the 51-residue oligodeoxyribonucleotide whose sequence is given above with the Pharmacia Gene Assembler DNA synthesis apparatus. The trityl coupling efficiency for the synthesis is given in Table IV.

(d) Use of Solid Support of Example 8 For Synthesis With Pharmacia Synthesis Apparatus

The solid support of Example 8, above, was used for synthesis of the same 51-residue oligodeoxyribonucleotide with the Pharmacia Gene Assembler DNA synthesis apparatus.

(e) Synthesis Of Controlled Pore Glass (500 Å Porosity) (Control) With Biosearch Synthesis Apparatus

As a control, controlled pore glass (500 Å porosity) was used for synthesis of the 51-residue oligodeoxyribonucleotide whose sequence was given above with the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency for this synthesis is given in Table V.

(f) Use of Solid Support of Example 1 For Synthesis With Biosearch Synthesis Apparatus

The solid support of Example 1, above, was used for synthesis of the 51-residue oligodeoxyribonucleotide whose sequence is given above with the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency is shown in Table V.

(g) Use of Solid Support of Example 7 For Synthesis With Biosearch Synthesis Apparatus

The solid support of Example 7 was used for synthesis of the 51-residue oligodeoxyribonucleotide whose sequence is given above with the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency is shown in Table V.

(h) Use of Solid Support of Example 8 For Synthesis With Biosearch Synthesis Apparatus

The solid support of Example 8, above, was used for synthesis of the 51-residue oligodeoxyribonucleotide whose sequence is given above on the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency for this synthesis is given in Table V.

(i) Use of Solid Support of Example 8 For Synthesis With Biosearch Synthesis Apparatus

The solid support of Example 8, above, was used for synthesis of the 51-residue oligodeoxyribonucleotide whose sequence is given above on the Biosearch 8750 DNA synthesis

apparatus. The trityl coupling efficiency for this synthesis is given in Table V.

Example 13

DNA Synthesis of 70-Mer Using Derivatized Solid Support

A 70-residue oligodeoxyribonucleotide, with the sequence of 5'-GATGCCAGTTCGGTCATCCGATGCTCGGTCACGGAACTGTCAACGGTACCTACTTGTCG TAACGTAGGAT-3' (SEQ ID NO: 5), was synthesized using the solid supports described above with various DNA synthesis apparatus.

(a) Use Of Controlled Pore Glass (1000 Å Porosity) (Control) With Pharmacia Synthesis Apparatus

As a control, the 70-residue oligodeoxyribonucleotide whose sequence is given above was synthesized using controlled pore glass (1000 Å porosity), on the Pharmacia Gene Assembler DNA synthesis apparatus. The trityl coupling efficiency for this synthesis is given in Table IV.

(b) Use of Solid Support of Example 1 With Pharmacia Synthesis Apparatus

The solid support of Example 1 was used with the Pharmacia Gene Assembler DNA synthesis apparatus for synthesis of the 70-residue oligodeoxyribonucleotide whose sequence is given above. The trityl coupling efficiency for this synthesis is shown in Table IV.

(c) Use of Solid Support of Example 5 For Synthesis With Pharmacia Synthesis Apparatus

The solid support of Example 5 was used for synthesis of the 70-residue oligodeoxyribonucleotide whose sequence is given above with the Pharmacia Gene Assembler DNA synthesis apparatus. The trityl coupling efficiency for the synthesis is given in Table IV.

(d) Use of Controlled Pore Glass (1000 Å Porosity) (Control) With Biosearch Synthesis Apparatus

As a control, the 70-residue oligodeoxyribonucleotide whose sequence is given above was synthesized using controlled pore glass (1000 Å porosity) with the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency for the synthesis is given in Table V.

(e) Use of Solid Support of Example 7 For Synthesis With Biosearch Synthesis Apparatus

The solid support of Example 7, above, was used for synthesis of the 70-residue oligodeoxyribonucleotide whose sequence is given above with the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency is shown in Table V.

Example 14

DNA Synthesis of 101-Mer Using Derivatized Solid Support

A oligodeoxyribonucleotide of 101 residues, with the sequence of 5'-
AACGTCGGTAACGTACACGGTAGCTACGGACACCGTGGCAATACGACAGGTAACCTGTGGAA
CGTACACGGAAGAGACTAGGGATGGGAGTACGGATGGGT-3' (SEQ ID NO: 6), was synthesized using various solid supports as described above and various DNA synthesis apparatuses.

(a) Use Of Controlled Pore Glass (1000 Å Porosity) (Control) With Pharmacia Synthesis Apparatus

As a control, the 101-residue oligodeoxyribonucleotide whose sequence is given above was synthesized using controlled pore glass (1000 Å porosity), with the Pharmacia Gene Assembler DNA synthesis apparatus. The trityl coupling efficiency is shown in Table IV.

(b) Synthesis Using Solid Support of Example 1 With Pharmacia Synthesis Apparatus

The 101-residue oligodeoxyribonucleotide whose sequence is given above was synthesized using the solid support of Example 1 with the Pharmacia Gene Assembler DNA synthesis apparatus. The trityl coupling efficiency for this synthesis is given in Table IV.

(c) Synthesis With Solid Support of Example 5 Using Pharmacia Synthesis Apparatus

The 101-residue oligodeoxyribonucleotide whose sequence is given above was synthesized using the solid support of Example 5 with the Pharmacia Gene Assembler DNA synthesis apparatus. The trityl coupling efficiency for the synthesis is given in Table IV.

(d) Synthesis Using Solid Support of Example 6 with Pharmacia Synthesis Apparatus

The 101-residue oligodeoxyribonucleotide whose sequence is given above was synthesized using the solid support of Example 6 with the Pharmacia Gene Assembler DNA synthesis apparatus.

(e) Use of Solid Support of Example 8 With Pharmacia Synthesis Apparatus

The 101-residue oligodeoxyribonucleotide whose sequence is given above was synthesized using the solid support of Example 8 with the Pharmacia Gene Assembler DNA synthesis apparatus.

(f) Synthesis With Controlled Pore Glass (1000 Å Porosity) (Control) With Biosearch Synthesis Apparatus

As a control, the 101-residue oligodeoxyribonucleotide whose sequence is given above was synthesized using controlled pore glass (1000 Å porosity) with the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency is shown in Table V.

(g) Synthesis Using Solid Support of Example 7 With Biosearch Synthesis Apparatus

The 101-residue oligodeoxyribonucleotide whose sequence is given above was synthesized using the solid support of Example 7, above, with the Biosearch 8750 DNA synthesis apparatus. The trityl coupling efficiency for the synthesis is shown in Table V.

Capillary gel electropherograms of the synthesized DNA of Examples 10(d), 11(c), 11(d), 11(e), 11(i), 11(j) (two batches), 11(k), 11(l), 11(m), 11(n), 11(o), 11(p), 11(q), 12(a), 12(b), 12(c), 12(d), 12(e), 12(f), 12(g), 12(h), 12(i), 13(a), 13(b), 13(c), 13(d), 14(a), 14(b), 14(c), 14(d), and 14(e) indicated that the synthesized DNA was at least of substantially equivalent purity to that obtained by synthesis

of the same DNA sequence on a controlled pore glass solid support.

Example 15

Polyacrylamide Slab Gel Electrophoresis

Polyacrylamide slab gel electrophoresis of the synthesized oligodeoxyribonucleotides was performed according to the following protocol. A 22 cm x 16.5 cm denaturing gel was prepared by adding 107.3 ml of water to a 100 g bottle of premixed acrylamide/methylenebisacrylamide (29:1) to make a 50% stock solution. The following were mixed: (1) 20 ml of the 50% acrylamide stock solution; (2) 22.5 g urea; and (3) 5 ml of 10 x TBE (0.9 mole/l Tris-borate, pH 8.0, 0.02 mole/l EDTA). The volume was brought to 50 ml with water, and the solution was heated and stirred to dissolve the urea. To initiate polymerization, 20 mg of ammonium persulfate and 20 µl N,N,N,N,-tetramethylenediamine (TEMED) was added. The gel was poured into the plates, and allowed to polymerize for 1 hour. The gel was then prerun with 1 x TBE at 20 mA for 1 hour. The appropriate quantity of oligodeoxyribonucleotide (0.2-1 A_{260} unit) was mixed in 10 µl of water with 10 µl of 10 mole/l urea. The resulting 20-µl mixture was loaded onto the gel and run at 28 mA for 2-4 hours, depending on the length of the oligodeoxyribonucleotide. The oligodeoxyribonucleotide bands were visualized by UV shadowing on a thin layer chromatography fluorescent plate or by staining with ethidium bromide, as indicated below. The following slab gels were run:

Plate I (Figure 9: samples from Examples 10(a), 10(b), 10(c), 11(a), 11(b), 11(f), 11(o), 11(p), 11(p) (2nd batch), and 11(q)).

Plate II (Figure 10: samples from Examples 11(a), 11(b), 11(c), 11(d), and 11(e)).

Plate III (Figure 11: samples from Examples 12(f), 12(g), 11(i), 11(j), 11(j) (2nd batch), 11(j) (3rd batch), and 11(k)).

Plate IV (Figure 12: samples from Examples 14(a), 14(b), 14(c), 13(a), 13(b), 13(c), 12(a), 12(b) and 12(c)).

Plate V (Figure 13: samples from Examples 10(a), 10(b), 10(d), 10(c), 11(a), 11(b), 11(d), and 11(c)).

Plate VI (Figure 14: samples from Examples 11(a), 11(h), 11(a), and 11(g)).

Plate VII (Figure 15: samples from Examples 11(e), 11(h), 11(i), 11(l), 11(n), 11(i), and 11(n)).

Plate VIII (Figure 16: samples from Examples 12(a), 12(e), 12(b), and 12(d)).

Plate IX (Figure 17: samples from Examples 14(a), 14(b), and 14(d)).

Plate X (Figure 18: samples from Examples 14(h), 14(g), 13(e), 13(d), 12(m), 12(i)).

Plate X was done with ethidium bromide staining; the others were done with fluorescent visualization.

Example 16

Reverse Phase High Pressure Liquid Chromatography

Reverse phase high pressure chromatography separates the desired oligodeoxyribonucleotide, which is expected to carry a lipophilic dimethoxytrityl (DMT group) from failed partial sequences that are not expected to carry the DMT group. The oligodeoxyribonucleotides, cleaved and deprotected from the solid support using ammonia, were directly injected into the HPLC column. The HPLC column used was C_{18} Ultrasphere (Beckman Instruments, Fullerton, California), 5 μ particles, 4.6 mm x 25 cm. Buffers used were 0.1 mole/l ammonium acetate, pH 6.9 (Buffer A) and acetonitrile (Buffer B). The

elution program, at a flow rate of 1 ml/min, was a gradient to 15% Buffer B from zero time to 20 minutes, a gradient to 25% Buffer B from 20 minutes to 25 minutes, a gradient to 50% Buffer B from 25 minutes to 27 minutes, 50% Buffer B from 27 minutes to 30 minutes, and 0% Buffer B from 30 to 35 minutes.

Samples of synthesis products from the following examples were subjected to reverse phase HPLC: 9(c), 9(d), 9(e); all 24-mers synthesized in Example 10; and 11(a), 11(b), and 11(f).

The HPLC results showed that the nucleotides synthesized were substantially free of partial sequences, confirming the data from capillary gel electrophoresis and trityl coupling efficiency.

Example 17
Composition Analysis of Synthesized Nucleotides By Enzyme Digestion

To determine the composition of a number of the synthesized oligodeoxyribonucleotides, they were subjected to enzyme digestion and the products of digestion were separated by HPLC. The two enzymes used were phosphodiesterase I reconstituted with 5 ml of 40 mmole/l Tris, 10 mmole/l $MgCl_2$, pH 7.5) and alkaline phosphatase from Escherichia coli, at 200 units/1.8 ml. The assay used 0.01 units of phosphodiesterase I and 0.2 units of alkaline phosphatase per assay, which was 25 µl of phosphodiesterase and 2 µl of phosphatase per assay. The required quantities of enzyme were added to 1 to 2 A_{260} units of the oligonucleotides. The reactions were incubated at room temperature for 30 minutes to 2 hours. Subsequently, 0.5-1 A_{260} units of the digestion mixture were injected onto a

reverse phase HPLC column. The same column and elution conditions were used as in Example 16.

Aliquots of nucleotides synthesized in the following examples were subjected to composition analysis by enzyme digestion: 10(a), 10(b), 10(c), 10(d), 11(a), 11(b)(2 batches), 11(c), 11(d), 11(e), 11(f), 11(g), 11(h), 11(i), 11(j), 11(k), 11(l), 11(n), 11(o), 11(p)(2 batches), 11(q), 12(a), 12(b), 12(e), 12(f), 12(g), 13(a), 13(b), 14(a), and 14(b). The composition analyses are shown in Tables VII through IX. The agreements with theoretical composition values are quite good, especially considering that no purification was done of the synthesized oligonucleotides before the compositions were determined.

TABLE VII

COMPOSITION ANALYSIS OF CRUDE OLIGONUCLEOTIDES SYNTHESIZED ON PHARMACIA GENE ASSEMBLER

Solid Support of Example No.:	Length of DNA Synthesized:				
	24 Theo./Obs.	36 Theo./Obs.	51 Theo./Obs.	70 Theo./Obs.	101 Theo./Obs.
CPG-Thymidine (Control)	A 6 5.97 C 8 7.65 G 5 5.93 T 5 4.45	A 9 13.00 C 9 7.29 G 8 7.23 T 9 7.48	A 10 9.93 C 15 14.1 G 13 12.7 T 13 14.28	A 15 16.32 C 18 15.48 G 19 21.80 T 18 16.38	A 30 32.4 C 20 15.83 G 35 34.58 T 16 18.19
Ex. 1: Fractogel - 65F-1 Thymidine	A 6 6.14 C 8 7.50 G 5 5.88 T 5 4.48	A 9 9.06 C 9 9.39 G 8 7.66 T 9 8.88	A 10 9.90 C 15 14.16 G 13 12.54 T 13 14.40	A 15 15.74 C 18 16.33 G 19 19.49 T 18 18.52	A 30 28.26 C 20 17.79 G 35 36.58 T 16 18.37
Ex. 1 (2nd Batch): Fractogel - 65F-1 Thymidine		A 9 9.26 C 9 8.48 G 8 8.04 T 9 9.21			
Ex. 2: Fractogel - 65F-1 Cytidine		A 9 10.29 C10 9.17 G 8 9.17 T 8 6.37			
Ex. 3: Fractogel - 65-F-1 Guanosine		A 9 8.52 C 9 8.84 G 9 9.73 T 8 7.90			
Ex. 4: Fractogel - 65-F-1 Adenosine		A10 11.09 C 9 8.28 G 8 8.79 T 8 6.82			
Ex. 5: Fractogel - 65M Thymidine	A 6 5.58 C 8 7.84 G 5 5.9 T 5 4.69	A 9 9.20 C 9 8.78 G 8 8.00 T 9 9.02			
Ex. 6: Fractogel - 65F-2	A 6 6.03 C 8 7.48 G 5 5.85 T 5 4.64	A 9 9.32 C 9 8.11 G 8 9.33 T 9 8.24			
Ex. 8: Fractogel - 65F-3 Thymidine		A 9 9.14 C 9 8.25 G 8 8.34 T 9 9.26	A 10 10.57 C 15 13.92 G 13 15.46 T 13 11.06		

TABLE VIII

COMPOSITION ANALYSIS OF CRUDE OLIGONUCLEOTIDES SYNTHESIZED ON BIOSEARCH 8750

Solid Support of Example No.:	Length of DNA Synthesized			
	35		51	
	Theo.	Obs.	Theo.	Obs.
CPG - Thymidine (Control)	A 9	8.98	A 10	9.16
	C 9	8.59	C 15	13.04
	G 8	8.21	G 13	14.8
	T 9	9.21	T 13	13.99
Ex. 1: Fractogel - 65F -1 Thymidine	A 9	8.8	A 10	9.42
	C 9	9.011	C 15	14.00
	G 8	8.01	G 13	12.71
	T 9	9.26	T 13	14.87
Ex. 1 (2nd Batch): Fractogel - 65F-1 Thymidine	A 9	8.55		
	C 9	8.50		
	G 8	9.47		
	T 9	8.47		
Ex. 5: Fractogel - 65M Thymidine	A 9	9.47		
	C 9	7.38		
	G 8	9.83		
	T 9	8.32		
Ex. 6: Fractogel - 65F-2 Thymidine	A 9	9.02		
	C 9	8.23		
	G 8	9.14		
	T 9	8.51		
Ex. 8: Fractogel - 65F-3 Thymidine	A 9	9.18		
	C 9	8.60		
	G 8	7.76		
	T 9	9.46		

TABLE IX

COMPOSITION ANALYSIS OF CRUDE OLIGONUCLEOTIDES SYNTHESIZED ON ABI PCR MATE

Solid Support of Example No.:	DNA Synthesized (35 Bases)	
	Theoretical	Observed
CPG - Thymidine (Control)	A 6 C 9 G 8 T 9	9.23 8.16 9.61 8.00
Ex. 1: Fractogel - 65F-1 Thymidine	A 9 C 9 G 8 T 9	9.62 8.00 10.03 7.35
Ex. 1 (2nd Batch): Fractogel -65F-1 Thymidine	A 9 C 9 G 8 T 9	9.13 7.94 9.88 8.04
Ex. 5: Fractogel - 65M Thymidine	A 9 C 9 G 8 T 8	8.69 8.40 9.77 8.14

Example 18

Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) was run with the template M13mp18 RF I DNA (New England Biolabs, Beverly, Mass.) using an 18-mer and a 22-mer as primer. The sequences were 5'-CGCCAGGGTTTTCCCAGT-3' (SEQ ID NO: 7) for the 18-mer and 5'-TTCTGGCGTACCGTTCCTGTCT-3' (SEQ ID NO: 8) for the 22-mer. These primers were synthesized on controlled pore glass supports and on the support of Example 1 and were purified before use by Sep Pak™ cartridge (Waters Chromatography Division of Millipore, Milford, Mass.). The PCR was performed according to the published instructions for the Perkin-Elmer-Cetus amplification reagent kit (Perkin-Elmer-Cetus, Norwalk, Connecticut). Initial melting was at 95°C for 7 minutes, and 25 cycles were then run on the DNA thermal cycler with the following cycle profile: segment 1, 94°C for 1 second; segment 2, 94°C for 1 minute; segment 3, 37°C for 1 second; segment 4, 37°C for 2 minutes; segment 5, 72°C for 1 second; and segment 6, 72°C for 3 minutes. The 957 base pair PCR products were electrophoresed on a 1% agarose gel in TAE (0.04 mole/l Tris-acetate, 0.001 mole/l EDTA), and stained with ethidium bromide, as shown in Figure 19. In Figure 19 lane 1 is the product from the CPG-synthesized primers, lanes 2 and 3 are the products from the primers synthesized by the solid support of Example 1, lane 4 is a gel marker consisting of bacteriophage λ DNA digested with the restriction endonuclease Hind III, showing 2322 and 2027 base pair markers, and lane 5 is a gel marker consisting of plasma pBR 322 DNA digested with the restriction endonuclease Hinf I, showing 1632 and 506 base pair markers. The PCR products from the oligonucleotides synthesized on the solid support of Example 1 were substantially identical to those obtained with the oligonucleotides synthesized on controlled pore glass.

Example 19
DNA Sequencing

DNA sequencing was performed using the same purified 18-mer primer synthesized in Example 18. The template was M13mp18 single-stranded DNA (New England Biolabs). The published protocol using α-[^{35}S]-dATP was followed for chain-termination sequencing using the Sequenase™ enzyme (United States Biochemical, Cleveland, Ohio). The nucleotides synthesized using the primers originally synthesized with controlled pore glass and the solid support of Example 1 were compared by gel electrophoresis (Figure 20). The results were substantially identical.

Example 20
5'-Kinase Reaction

A 22-mer as synthesized in Example 18 for the polymerase chain reaction on controlled pore glass and the solid support of Example 1 were purified by Sep Pak (Waters Chromatography Division of Millipore, Milford, Mass.) C_{18} cartridge before the kinase reaction by diluting the oligonucleotide 1:2 into 0.5 mole/l ammonium acetate, loading it onto the cartridge, washing the cartridge with water, diluting the oligonucleotide with 60% methanol in water, and evaporating the methanol:water solvent. The oligonucleotides were then resuspended in water to a concentration of 1 μg/μl.

For the kinasing procedure, 10μ of the oligonucleotide was mixed with 10 μl of 10 x kinase buffer (0.5 mole/l Tris-HCl, pH 7.6, 0.1 mmole/l $MgCl_2$, 50 mmole/l dithiothreitol, and 1 mmole/l EDTA), 10μl of ATP (10 mmole/l), and 30 units T4 polynucleotide kinase (Boehringer Mannheim, Mannheim, Germany) and the volume was brought to 100 μl with water. The reaction mixtures were incubated at 37°C for 45

minutes, then heated at 75°C for 5 to 10 minutes to inactivate the enzymes. The reaction mixtures were analyzed by capillary gel electrophoresis as described above with injection for 10 seconds at 10 kV and separate at 11 kV for 45 minutes at 20°C. The phosphorylated oligonucleotides were differentiated from non-phosphorylated oligonucleotides by spiking the kinase reaction mix 1:2 with 50 μg oligonucleotides and analyzed on capillary gel electrophoresis in the same manner. Both the oligonucleotides synthesized on the solid support of Example 1 and the controlled pore glass were phosphorylated at the 5'-ends quantitatively.

Example 21
3'-Terminal Transferase Extension

To determine the availability of the 3'-ends of the oligodeoxyribonucleotides for extension by transferase, transferase reactions were performed. The following were mixed: 2.5 A_{260} of the oligonucleotide in 150 μl water, 5 mg TTP (Sigma Chemical Company, St. Louis, Missouri), 5 μl terminal deoxynucleotidyl transferase (15 units/μl) (Bethesda Research Labs, Bethesda, Maryland), and 50 μl tailing buffer (Bethesda Research Labs). The reactions were incubated overnight at 37°C. The reacted nucleotides were purified by Sep Pak C^{18} cartridge by diluting the reaction mixture 1:2 in 0.5 mmole/l ammonium acetate, loading on the cartridge, washing the cartridge with water, and eluting the oligonucleotides with 60% methanol in water. The eluted oligonucleotides were analyzed by capillary get electrophoresis in the same manner as described above. Both the oligonucleotides synthesized on the controlled pore glass and on the solid support of Example 1 were substantially quantitatively extended at the 3'-end by the terminal transferase.

Although the present invention has been described in considerable detail with regard to certain preferred versions thereof, other versions are possible. Therefore, the spirit and scope of the appended claims should not be limited to the descriptions of the preferred versions contained herein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Reddy, Parameswara M
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(ii) TITLE OF INVENTION: Derivatized Organic Solid Support for Nucleic Acid Synthesis

(iii) NUMBER OF SEQUENCES: 8

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/___,___
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTAAATGCC ATCGACT 17

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATTGAGAAAG CGCCACGCTT CCCT 24

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATGCCAGTT CGGTCATACA CGTAGTACTA CGACT 35

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCCATGGCAA CTGTCAAGGC ACTGGCTCGT AGCCTACTGG CTTGACCGTA T 51

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATGCCAGTT CGGTCATCCG ATGCTCGGTC ACGGAACTGT CAACGGTACC TACTTGTCGT 60

AACGTAGGAT 70

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 101 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AACGTCGGTA ACGTACACGG TAGCTACGGA CACCGTGGCA ATACGACAGG TAACCTGTGG 60

AACGTACACG GAAGAGACTA GGGATGGGAG TACGGATGGG T 101

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGCCAGGGTT TTCCCAGT 18

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTCTGGCGTA CCGTTCCTGT CT 22

What is claimed is:

1. A solid-phase nucleotide synthesis intermediate comprising:

(a) a particulate support comprising a porous polymer whose backbone comprises optionally substituted acrylate or methacrylate moieties;

(b) a nucleoside; and

(c) a linker having a first and a second end, the first end being covalently attached to the particulate support and the second end being covalently attached to the nucleoside, the linker spacing the nucleoside at least 3 atoms away from the polymer.

2. The intermediate of claim 1 wherein the linker spaces the nucleoside from the polymer by about 6 atoms to about 36 atoms.

3. The intermediate of claim 1 wherein the linker comprises at least one aliphatic diamine, each aliphatic diamine optionally containing up to 2 heteroatoms replacing carbon atoms and selected from the group consisting of O and N, the heteroatoms being bonded solely to carbon and being separated from the terminal amino group of the diamine by at least one carbon atom, the aliphatic diamines having a total chain length of from about 6 to about 36 atoms, with the proviso that, if more than one diamine is present in the linker, the diamines are linked to each other by carbonyl groups.

4. The intermediate of claim 1 wherein the porous polymer is substantially free from pyranose or furanose moieties or derivatives of pyranose or furanose. moieties.

5. The intermediate of claim 1 wherein the nucleoside is a protected deoxyribonucleoside.

6. The intermediate of claim 5 wherein the protected deoxyribonucleoside is protected at its 5'-end.

7. The intermediate of claim 6 wherein the 5'-protected deoxyribonucleotide is an aryl ester.

8. The intermediate of claim 5 wherein the protected deoxyribonucleoside is protected at its 3'-end.

9. The intermediate of claim 1 wherein the nucleoside is a protected ribonucleoside.

10. The intermediate of claim 9 wherein the protected ribonucleoside is protected at its 5'-end.

11. The intermediate of claim 9 wherein the protected ribonucleoside is protected at its 3'-end.

12. A solid-phase nucleotide synthesis intermediate comprising:

(a) a porous solid-phase particulate support comprising a porous organic polymer, at least partially hydrophilic and having hydroxy residues, whose backbone comprises straight-chain optionally substituted acrylate or methacrylate moieties;

(b) a linear polymer having first and second ends, the linear polymer being covalently linked through the first end to the particulate support, the linear polymer comprising at least one aliphatic diamine, each aliphatic diamine optionally containing up to 2 heteroatoms replacing carbon atoms, the heteroatoms being selected from the group

consisting of O and N, the heteroatoms being bonded solely to carbon and being separated from the terminal amino group of the diamine by at least one carbon atom, the aliphatic diamines having a total chain length of from about 3 to about 36 carbon atoms, with the proviso that, if more than one diamine is present in the linker, the diamines are linked to each other by carbonyl groups, the linear polymer having an amino group at its second end available for reaction with an activated protected deoxyribonucleoside, the linkage between the particulate support and the first end of the linear polymer comprising a carbamate moiety; and

(c) a nucleoside linked to the second end of the linear polymer.

13. The intermediate of claim 12 wherein the nucleoside is an activated 5'-protected deoxyribonucleoside, the activated protected deoxyribonucleoside comprising an aryl ester, and wherein the carboxyl function of the aryl ester is linked in amide linkage to the amino group at the second end of the linear polymer.

14. The intermediate of claim 12 wherein the porous organic polymer comprises a methacrylate-vinylidene copolymer.

15. The intermediate of claim 12 wherein the linear polymer comprises only one aliphatic diamine.

16. The intermediate of claim 12 wherein the linear polymer comprises only two aliphatic diamines.

17. The intermediate of claim 12 wherein the linear polymer comprises only three aliphatic diamines.

18. The intermediate of claim 15 wherein the aliphatic diamine has a chain length of 6 carbons.

19. The intermediate of claim 15 wherein the aliphatic diamine has a chain length of 12 carbons.

20. The intermediate of claim 16 wherein each aliphatic diamine has a chain length of 6 carbons.

21. The intermediate of claim 17 wherein each aliphatic diamine has a chain length of 6 carbons.

22. A solid-phase nucleotide synthesis intermediate comprising:

(a) a porous solid-phase particulate support comprising a methacrylate-vinylidene copolymer;

(b) a polyethylene glycol linker having from about 2 to about 20 ethylene glycol moieties and having a first end and a second end, the first end covalently attached to the particulate support, the second end terminating in an amine moiety; and

(c) a nucleoside linked to the amine moiety at the second end of the polyethylene glycol linker by an amide linkage between the amine moiety and a carboxyl group in covalent linkage with the nucleoside.

23. The intermediate of claim 22 wherein the polyethylene glycol linker has from about 4 to about 7 ethylene glycol moieties.

24. The intermediate of claim 22 wherein the mean diameter of the particles of the porous particulate support is from about 30 microns to about 70 microns.

25. The intermediate of claim 22 wherein the nucleoside is a protected deoxyribonucleoside.

26. The intermediate of claim 25 wherein the protected nucleoside is protected at its 5'-end.

27. A solid-phase nucleotide synthesis intermediate comprising:

(a) a porous solid-phase particulate support comprising a porous organic polymer, at least partially hydrophilic and having hydroxy residues, whose backbone comprises straight-chain optionally substituted acrylate or methacrylate moieties;

(b) a linear polymer having first and second ends, the linear polymer being covalently linked through the first end to the particulate support, the linear polymer comprising at least one aliphatic diamine, each aliphatic diamine optionally containing up to 2 heteroatoms replacing carbon atoms and selected from the group consisting of O and N, the heteroatoms being bonded solely to carbon and being separated from the terminal amino group of the diamine by at least one carbon atom, the aliphatic diamines having a total chain length of from about 3 to about 36 carbon atoms, with the proviso that, if more than one diamine is present in the linker, the diamines are linked to each other by carbonyl groups, the linear polymer having an amino group at its second end available for reaction with a nucleoside, the linkage between the particulate support and the first end of the linear polymer comprising a spacer with the structure $-CH_2-CH(OCOCH_3)-CH_2-$, with the first amino group of the diamine moiety attached to the particulate support being substituted with an acetyl group; and

(c) a nucleoside linked to the second end of the linear polymer.

28. The intermediate of claim 27 wherein the nucleoside is an activated 5'-protected deoxyribonucleoside, the activated protected deoxyribonucleoside comprising an aryl ester, and wherein the carboxyl function of the aryl ester is linked in amide linkage to the amino group at the second end of the linear polymer.

29. The intermediate of claim 27 wherein the porous organic polymer comprises a methacrylate-vinylidene copolymer.

30. A nucleotide synthesis intermediate comprising:

(a) a macroreticular methacrylate-vinylidene copolymer having hydroxy residues;

(b) a diaminohexane moiety linked to a hydroxy residue of the copolymer through a carbonyl group bound to the first amino group of the diaminohexane moiety, forming a carbamate linkage; and

(c) a 5'-protected deoxyribonucleoside linked to the second amino group of the diaminohexane moiety through a succinyl group, thereby forming an amide linkage with the second amino group and an ester linkage between the succinyl group and the protected deoxyribonucleoside.

31. A nucleotide synthesis intermediate comprising:

(a) a macroreticular methacrylate-vinylidene copolymer having hydroxy residues;

(b) a diaminododecane moiety linked to a hydroxy residue of the copolymer through a carbonyl group bound to the first amino group of the diaminohexane moiety, forming a carbamate linkage; and

(c) a 5'-protected deoxyribonucleoside linked to the second amino group of the diaminododecane moiety through a succinyl group, thereby forming an amide linkage with the

second amino group and an ester linkage between the succinyl group and the protected deoxyribonucleoside.

32. A nucleotide synthesis intermediate comprising:

(a) a macroreticular methacrylate-vinylidene copolymer having hydroxy residues;

(b) a first diaminohexane moiety linked to a hydroxy residue of the copolymer through a carbonyl group bound to the first amino group of the diaminohexane moiety, forming a carbamate linkage;

(c) a second diaminohexane moiety whose first amino group is linked to the second amino group of the first diaminohexane moiety through a carbonyl group, forming a urea linkage; and

(d) a 5'-protected deoxyribonucleoside linked to the second amino group of the second diaminohexane moiety through a succinyl group, thereby forming an amide linkage with the second amino group and an ester linkage between the succinyl group and the protected deoxyribonucleoside.

33. A nucleotide synthesis intermediate comprising:

(a) a macroreticular methacrylate-vinylidene copolymer having hydroxy residues;

(b) a first diaminohexane moiety linked to a hydroxy residue of the copolymer through a carbonyl group bound to the first amino group of the diaminohexane moiety, forming a carbamate linkage;

(c) a second diaminohexane moiety whose first amino group is linked to the second amino group of the first diaminohexane moiety through a carbonyl group, forming a urea linkage;

(d) a third diaminohexane moiety whose first amino group is linked to the second amino group of the second

diaminohexane moiety through a carbonyl group, forming a urea linkage; and

(e) a 5'-protected deoxyribonucleoside linked to the second amino group of the third diaminohexane moiety through a succinyl group, thereby forming an amide linkage with the second amino group and an ester linkage between the succinyl group and the protected deoxyribonucleoside.

34. A nucleotide synthesis intermediate comprising:

(a) a macroreticular methacrylate-vinylidene copolymer having hydroxy residues;

(b) a polyethylene glycol linker having from 4 to 7 ethylene glycol moieties and having a first end and a second end, the first end covalently attached to the copolymer, the second end terminating in an amine moiety; and

(c) a 5'-protected deoxyribonucleoside linked to the amine moiety of the polyethylene glycol linker through a succinyl group, thereby forming an amide linkage with the amine moiety and an ester linkage between the succinyl group and the protected deoxyribonucleoside.

35. A nucleotide synthesis intermediate comprising:

(a) a macroreticular methacrylate-vinylidene copolymer having hydroxy residues;

(b) a diaminododecane moiety whose first amino group is linked to a hydroxy residue of the copolymer through a spacer with the structure $-CH_2-CH(OCOCH_3)-CH_2-$, with the first amino group of the diaminododecane moiety being substituted with an acetyl group; and

(c) a 5'-protected deoxyribonucleoside linked to the second amino group of the diaminododecane moiety through a succinyl group, thereby forming an amide linkage with the second amino group and an ester linkage between the succinyl group and the protected deoxyribonucleoside.

36. A process for producing a particulate porous solid-phase support derivatized with a nucleoside, comprising:

(a) coupling:

(i) a particulate support comprising a porous polymer whose backbone comprises optionally substituted acrylate or methacrylate moieties, with:

(ii) a linker having a first and a second end, the first end being covalently attached to the particulate support; and

(b) coupling the second end of the linker to a nucleoside, the linker spacing the nucleoside at least 3 atoms away from the polymer.

37. The process of claim 36 wherein the linker spaces the nucleoside from the polymer by about 6 atoms to about 36 atoms.

38. The process of claim 36 wherein the linker comprises at least one aliphatic diamine, each aliphatic diamine optionally containing up to 2 heteroatoms replacing carbon atoms and selected from the group consisting of O and N, the heteroatoms being bonded solely to carbon and being separated from the terminal amino group of the diamine by at least one carbon atom, the aliphatic diamines having a total chain length of from about 6 to about 36 atoms, with the proviso that, if more than one diamine is present in the linker, the diamines are linked to each other by carbonyl groups.

39. A process for producing a particulate porous solid-phase support derivatized with a nucleoside, comprising the steps of:

(a) coupling:

(i) a porous solid-phase particulate support comprising a porous polymer, at least partially hydrophilic and having hydroxy residues, whose backbone comprises straight-chain optionally substituted acrylate or methacrylate moieties, the porous polymer being extended with a linear polymer having first and second ends, the linear polymer being covalently linked through the first end to the porous polymer, the linear polymer comprising at least one aliphatic diamine, each aliphatic diamine optionally containing up to 2 heteroatoms replacing carbon atoms, the heteroatoms being selected from the group consisting of O and N, the heteroatoms being bonded solely to carbon and being separated from the terminal amino group of the diamine by at least one carbon atom, the aliphatic diamines having a total chain length of from about 6 to about 36 carbon atoms, with the proviso that, if more than one diamine is present in the linker, the diamines are linked to each other by carbonyl groups, the linear polymer having an amino group at its second end, with:

(ii) a nucleoside, such that the nucleoside is covalently linked to the amino group at the second end of the linear polymer;

(b) acylating terminal amino groups remaining uncoupled; and

(c) blocking hydroxy residues on the solid phase support.

40. The process of claim 39 wherein the porous polymer is substantially free of pyranose or furanose moieties or derivatives of pyranose or furanose moieties.

41. The process of claim 39 wherein the porous solid phase support is condensed with only one aliphatic diamine.

42. The process of claim 39 wherein the porous solid phase support is condensed with only two aliphatic diamines.

43. The process of claim 39 wherein the porous solid phase support is condensed with only three aliphatic diamines.

44. The process of claim 41 wherein the aliphatic diamine has a chain length of 6 carbons.

45. The process of claim 41 wherein the aliphatic diamine has a chain length of 12 carbons.

46. The process of claim 42 wherein each aliphatic diamine has a chain length of 6 carbons.

47. The process of claim 43 wherein each aliphatic diamine has a chain length of 6 carbons.

48. The process of claim 39 wherein the porous polymer is a methacrylate-vinylidene copolymer.

49. The process of claim 39 wherein the aliphatic diamine or diamines are straight-chain diamines.

50. A process for producing a particulate porous organic solid-phase support derivatized with a nucleoside, comprising the steps of:

(a) coupling:

(i) a porous solid-phase support comprising a methacrylate-vinylidene copolymer extended with polyethylene glycol, the polyethylene glycol having from about 2 to about 20 ethylene glycol moieties and containing a terminal amino

group, the terminal amino group being available for reaction with a nucleoside, with:

(ii) a nucleoside, such that the nucleoside is covalently linked to the terminal amino group;

thereby forming a solid support derivatized with the nucleoside; and

(b) acylating terminal amino groups remaining uncoupled.

51. The process of claim 50 wherein the polyethylene glycol has from 4 to 7 ethylene glycol moieties.

52. A process for producing a particulate porous organic solid-phase support derivatized with a nucleoside, comprising the steps of:

(a) condensing a porous solid-phase particulate support comprising a porous polymer, at least partially hydrophilic and having hydroxy residues, whose backbone comprises straight-chain optionally substituted acrylate or methacrylate moieties, with epichlorohydrin;

(b) condensing:

(i) the reaction product between the polymer and epichlorohydrin with:

(ii) at least one aliphatic diamine, each aliphatic diamine optionally containing up to 2 heteroatoms replacing carbon atoms, the heteroatoms being selected from the group consisting of O and N, the heteroatoms being bonded solely to carbon and being separated from the terminal amino group of the diamine by at least one carbon atom, the aliphatic diamines having a total chain length of from about 6 to about 36 carbon atoms, with the proviso that, if more than one diamine is present in the linker, the diamines are linked to each other by carbonyl groups, to form a modified solid phase support containing at least one diamine linked to the

solid support by a spacer with the structure $-O-CH_2-CHOH-CH_2-$, the modified solid phase support having a terminal amino group available for reaction with a nucleoside;

(c) reacting the modified solid phase support with a short-chain alkylamine;

(d) coupling the terminal amino group of the solid phase support with a nucleoside, thereby forming the solid support derivatized with the nucleoside; and

(e) acetylating terminal amino groups remaining uncoupled.

53. The process of claim 52 wherein the reaction product between the polymer and epichlorohydrin is condensed with only one diamine.

54. The process of claim 53 wherein the diamine has a chain length of 12 carbons.

55. The process of claim 53 wherein the polymer is a methacrylate-vinylidene copolymer.

56. A process for producing a particulate porous organic solid-phase support derivatized with a nucleoside, comprising the steps of:

(a) condensing:

(i) a porous solid-phase particulate support comprising a methacrylate-vinylidene copolymer having hydroxy residues, the particles of the porous support having a mean diameter of about 45 microns and having pores of a size that excludes proteins of molecular weight greater than about $5x10^6$, with:

(ii) one straight-chain aliphatic diamine having a chain length of 12 carbon atoms to form a modified solid-phase support extended by an aliphatic straight-chain

diamine moiety with a terminal amino group available for reaction with a nucleoside;

(b) coupling the terminal amino group with a nucleoside through an succinyl ester group coupled to the nucleoside so that the succinyl residue of the succinylated nucleoside is linked in amide linkage to the terminal amino group, thereby forming the solid support derivatized with the nucleoside, the quantity of protected deoxyribonucleoside derivatized per gram of solid-phase support being about 10 μmole/g to about 150 μmole/g;

(c) acetylating terminal amino groups remaining uncoupled in step (b); and

(d) blocking hydroxy residues on the solid-phase support.

57. A porous solid-phase support derivatized with a protected deoxyribonucleoside produced by the process of claim 36.

58. A porous solid-phase support derivatized with a protected deoxyribonucleoside produced by the process of claim 39.

59. A porous solid-phase support derivatized with a protected deoxyribonucleoside produced by the process of claim 50.

60. A porous solid-phase support derivatized with a protected deoxyribonucleoside produced by the process of claim 52.

61. A porous solid-phase support derivatized with a protected deoxyribonucleoside produced by the process of claim 56.

62. A process for synthesizing an oligodeoxyribonucleotide comprising the steps of:

(a) selecting the solid-phase nucleotide synthesis intermediate of claim 1, the nucleotide synthesis intermediate containing a 5'-protected deoxyribonucleoside;

(b) removing the protecting group of the 5'-protected deoxyribonucleoside attached to the solid support by treating the intermediate with acid to yield a free 5'-OH group;

(c) coupling the free 5'-OH group with a deoxyribonucleoside 3'-phosphoramidite having a 5'-protected hydroxyl to form a phosphite linkage between the 5'-carbon of the deoxyribonucleoside attached to the solid support and the deoxyribonucleoside residue of the phosphoramidite;

(d) oxidizing the phosphite linkage to a phosphotriester linkage to yield a dinucleotide attached to the solid support;

(e) acetylating remaining unreacted 5'-OH groups after the coupling steps;

(f) repeating, for each additional nucleotide residue added to the chain, the steps of: (b) removing the protecting group, (c) coupling the resulting free 5'-OH group with a deoxyribonucleoside 3'-phosphoramidite having a 5'-protected hydroxyl to form a phosphite linkage, (d) oxidizing the phosphite linkage to a phosphotriester linkage, and (e) acetylating remaining unreacted 5'-OH groups; and

(g) cleaving the oligodeoxyribonucleotide from the solid support.

63. A process for synthesizing an oligodeoxyribonucleotide comprising the steps of:

(a) selecting the solid-phase nucleotide synthesis intermediate of claim 1, the nucleotide synthesis intermediate containing a 5'-protected deoxyribonucleoside;

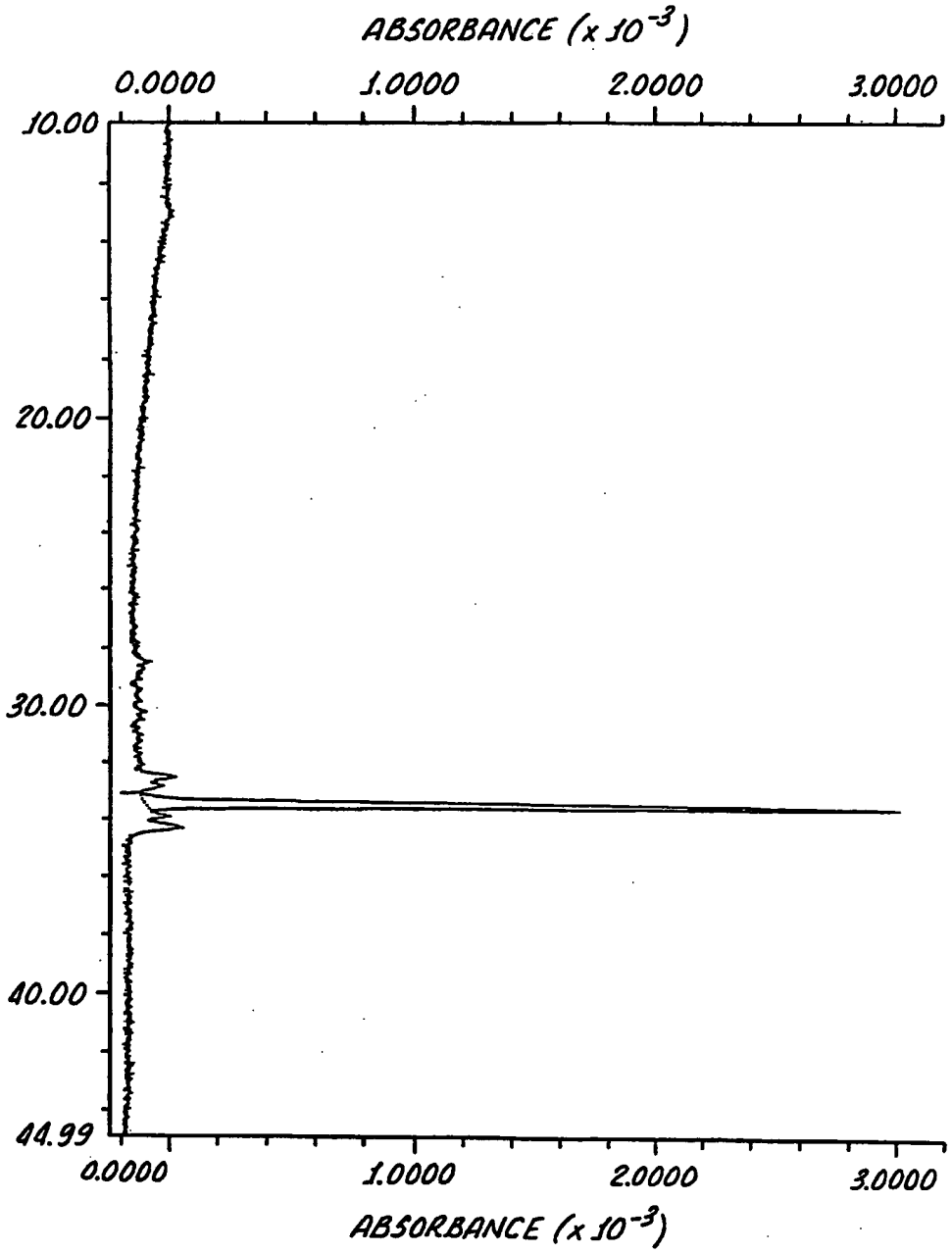
(b) removing the protecting group of the 5'-protected deoxyribonucleoside attached to the solid support by treating the intermediate with acid to yield a free 5'-OH group;

(c) coupling the free 5'-OH group with a 3'-O-phosphorylated deoxyribonucleoside having a protected 5'-OH group in the presence of a coupling agent and catalyst to form a dinucleotide attached to the solid support;

(d) optionally, acetylating remaining unreacted 5'-OH groups after the coupling steps;

(e) repeating, for each additional nucleotide residue added to the chain, the steps of (b) removing the protecting group, (c) coupling the free 5'-OH group with a 3'-O-phosphorylated deoxyribonucleoside, and (d) optionally, acetylating remaining unreacted 5'-OH groups; and

(f) cleaving the completed oligodeoxyribonucleotide from the solid support.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3.0000
10.00
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FIG. 1.

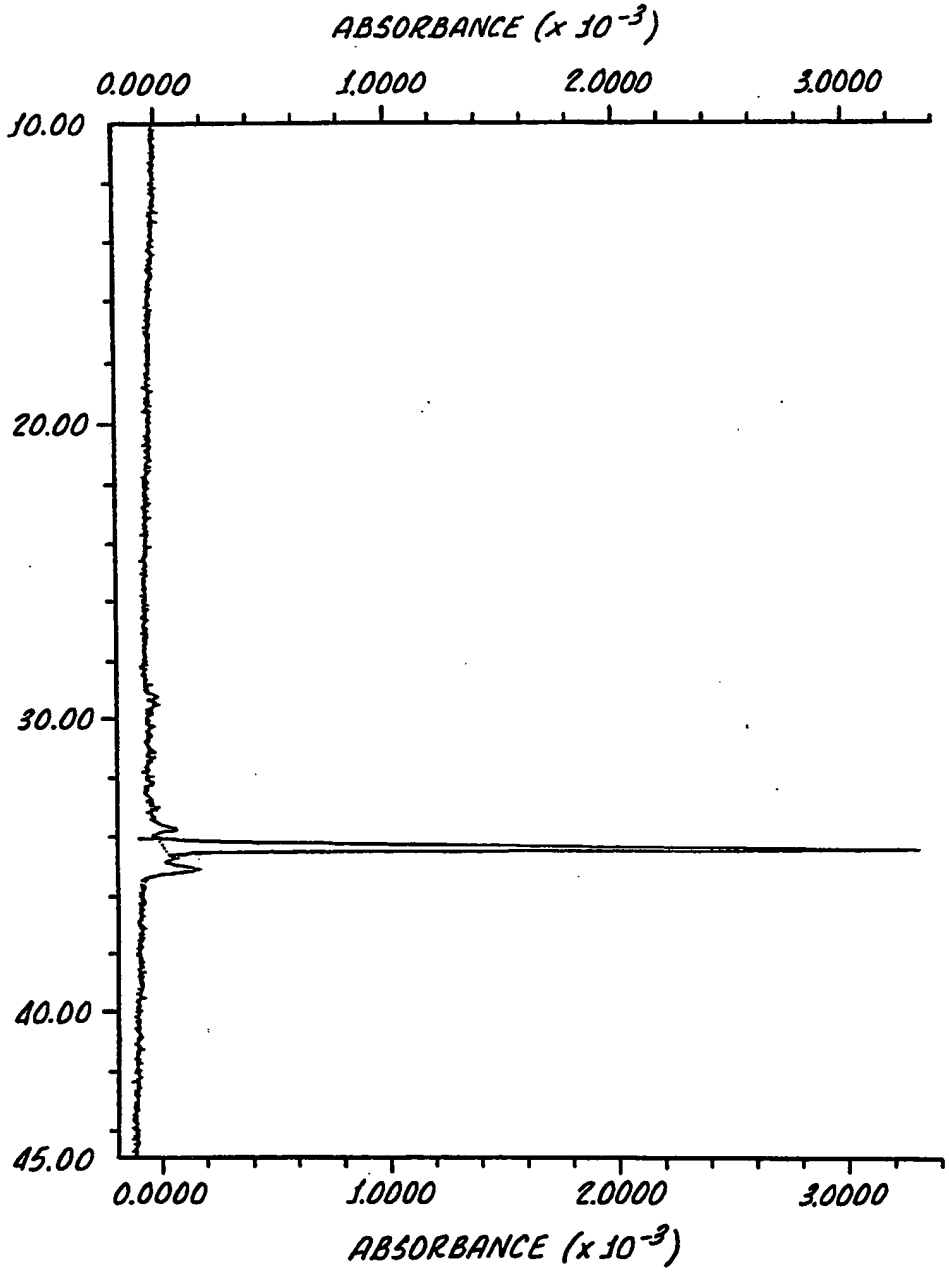
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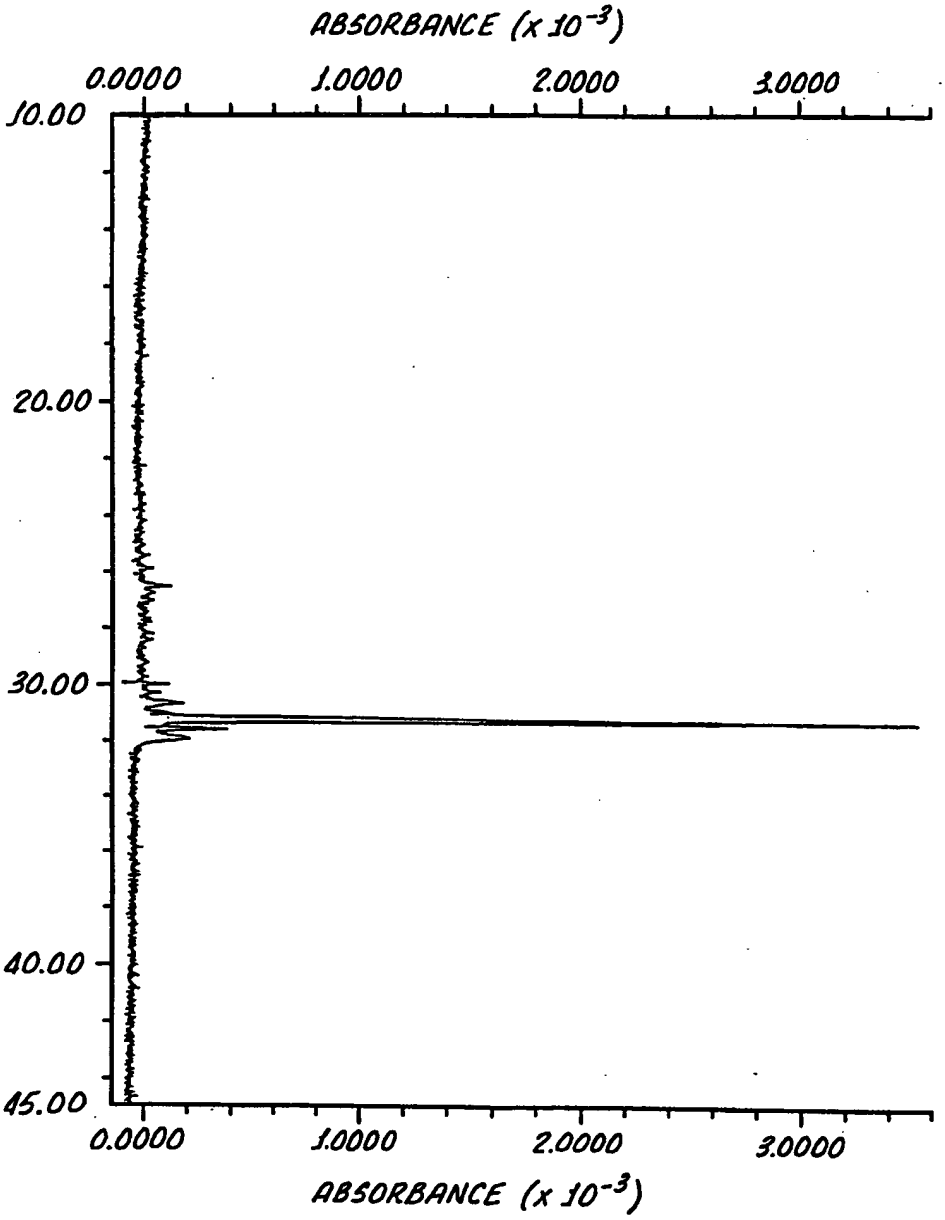
FIG. 2.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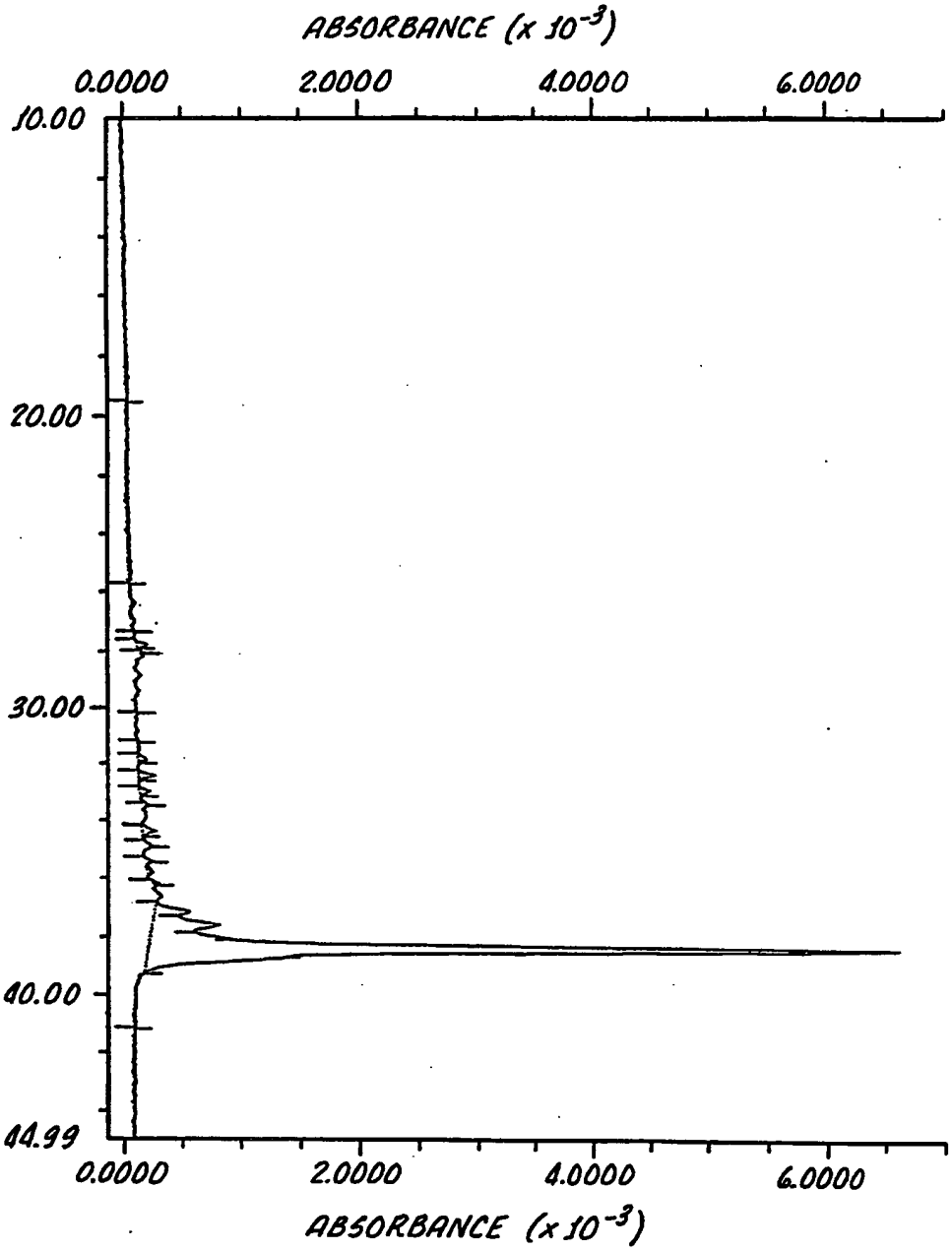
3.0000
ABSORBANCE (x 10^{-3})

FIG. 3.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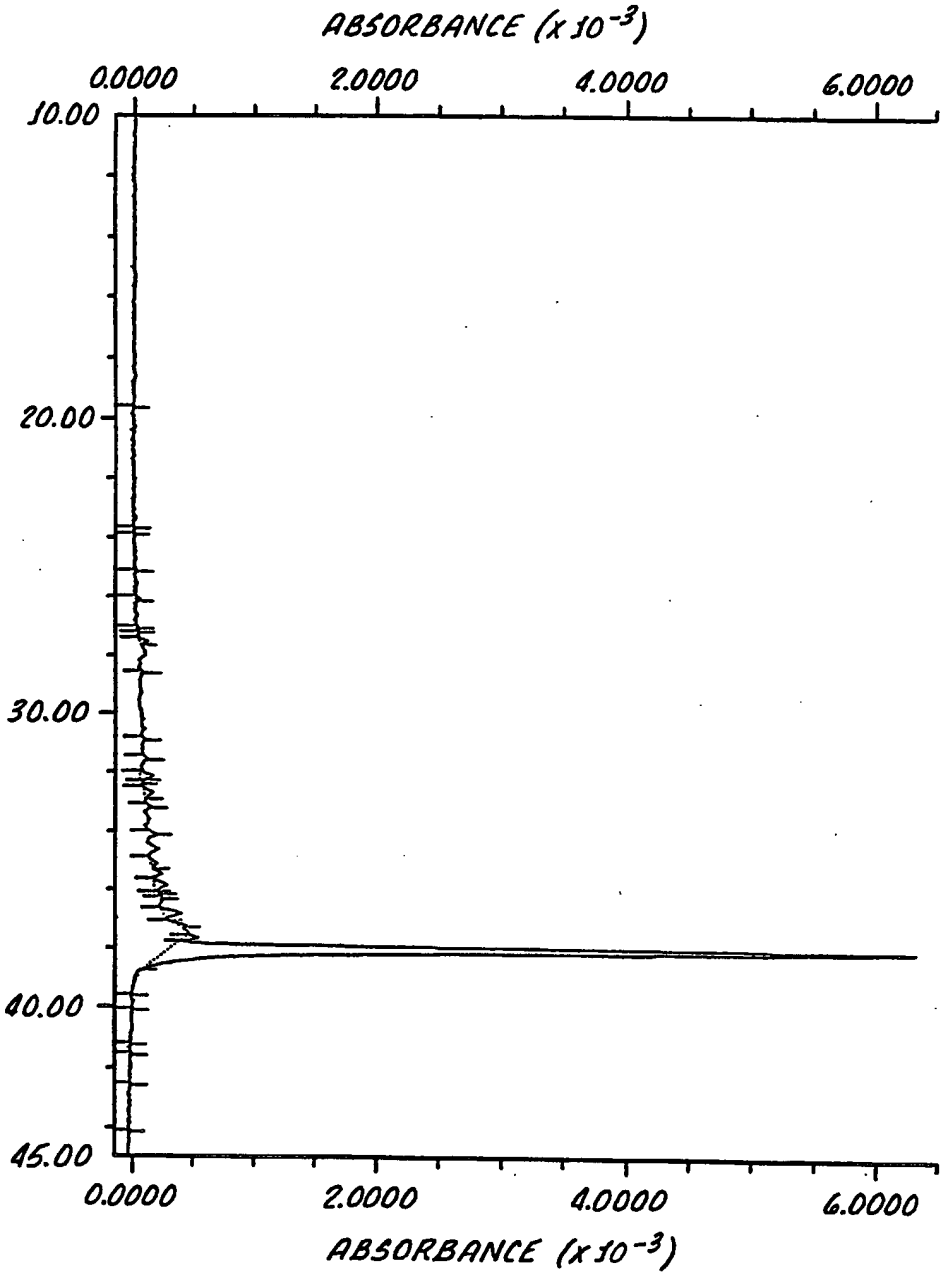
4.0000
6.0000
ABSORBANCE (x 10⁻³)

FIG. 4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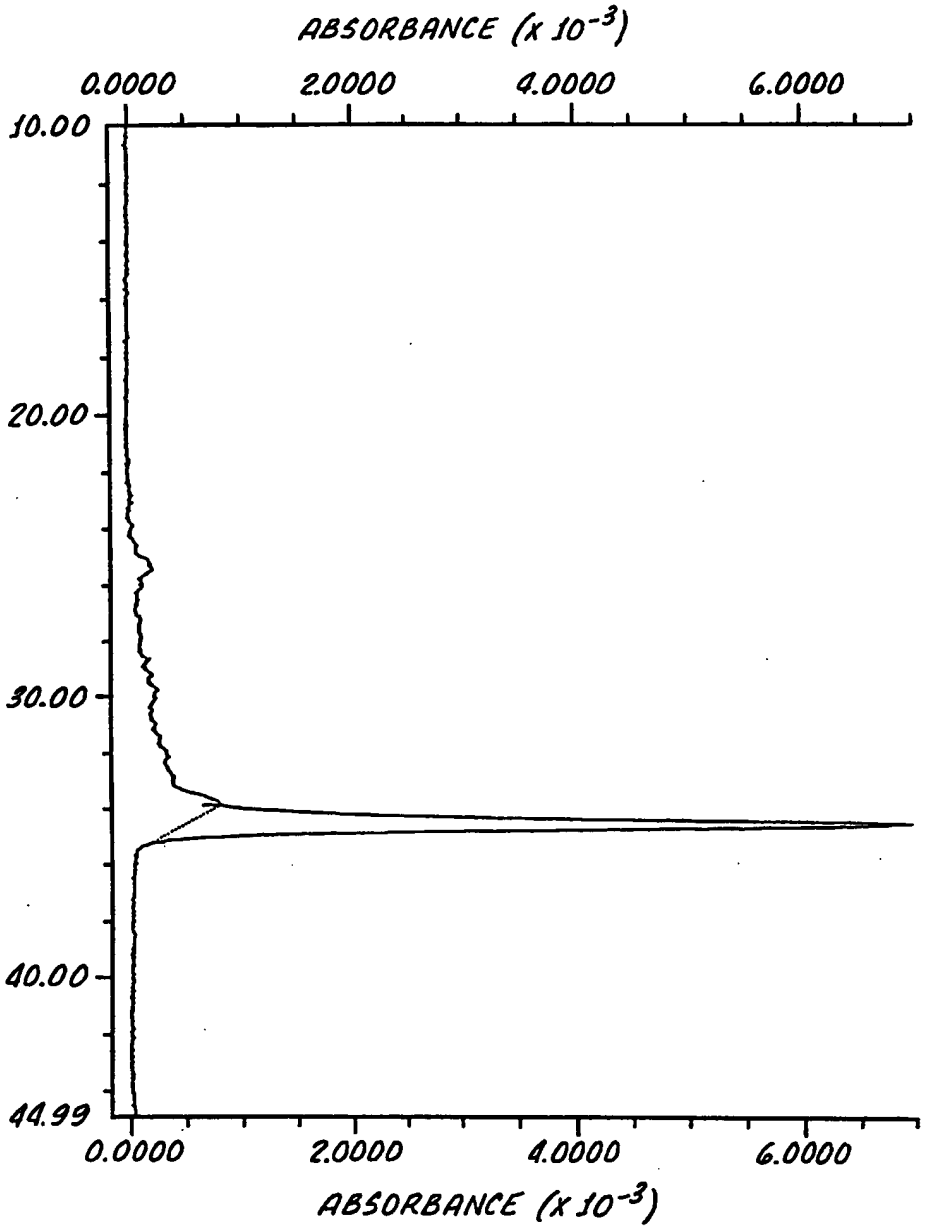
ABSORBANCE (x 10⁻³)

FIG. 5.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6.0000
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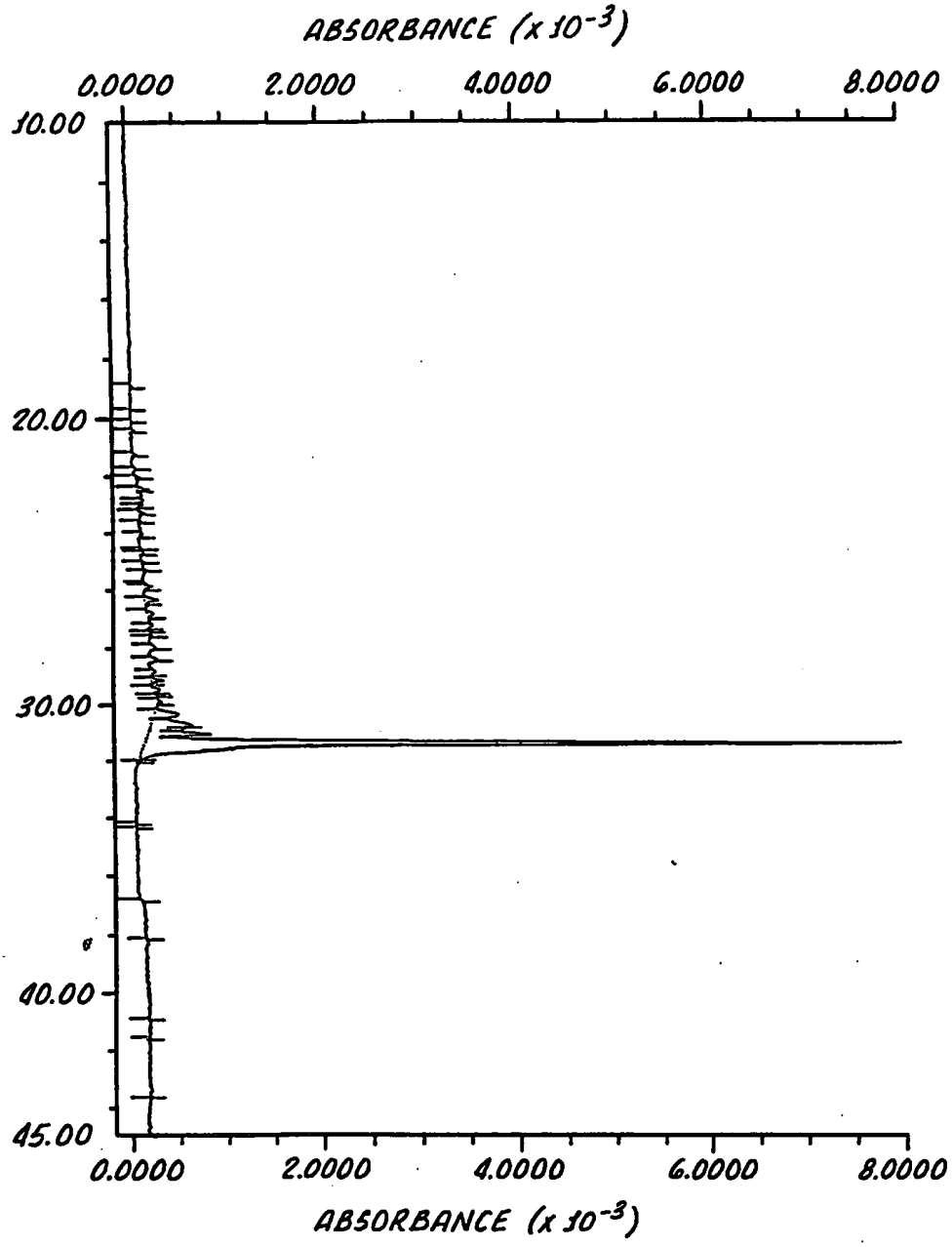
44.99
0.0000
2.0000
4.0000
6.0000
ABSORBANCE (x 10^{-3})

FIG. 6.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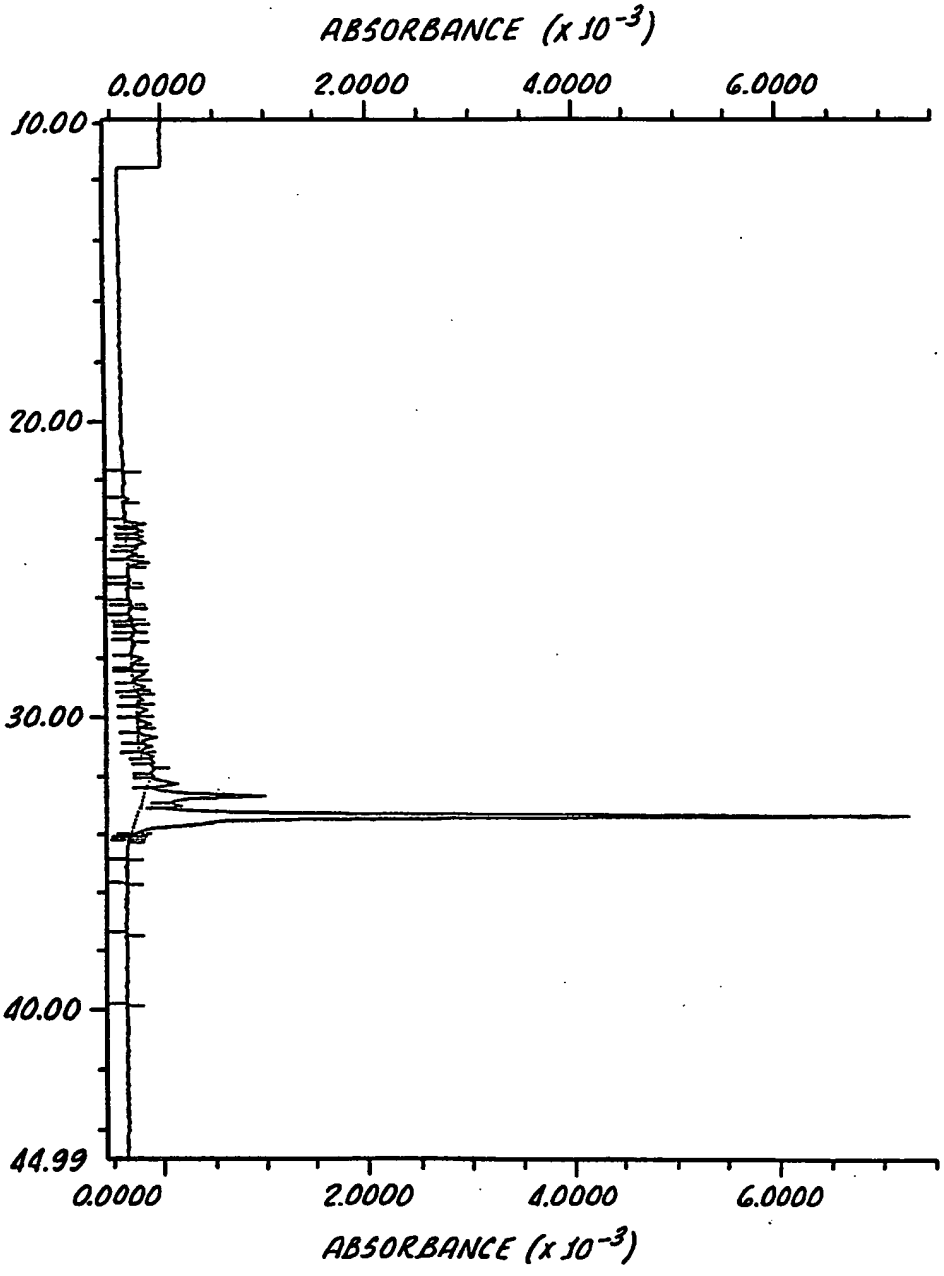
8.0000
ABSORBANCE (x 10⁻³)

FIG. 7.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4.0000
6.0000
10.00
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6.0000
ABSORBANCE (x 10⁻³)

FIG. 8.

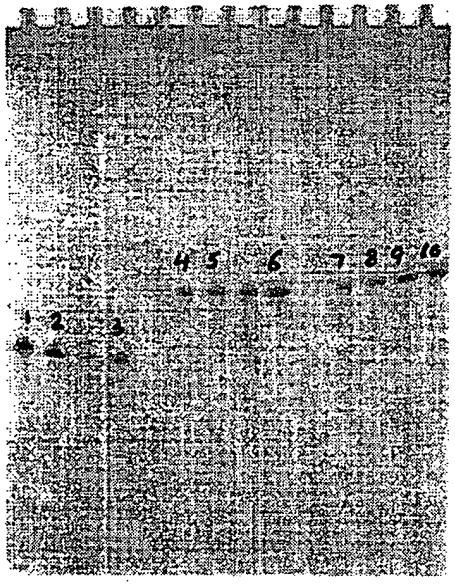

FIG. 9

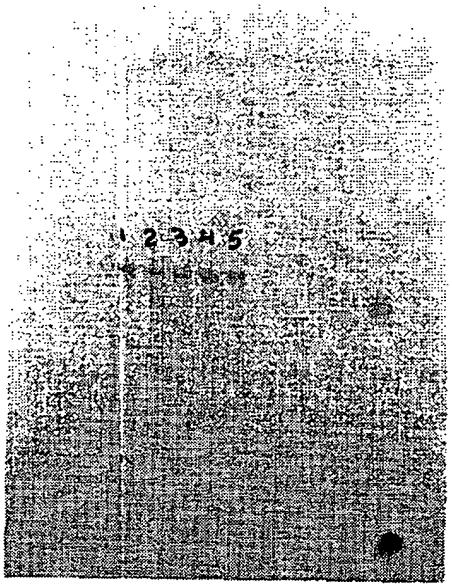

FIG.10

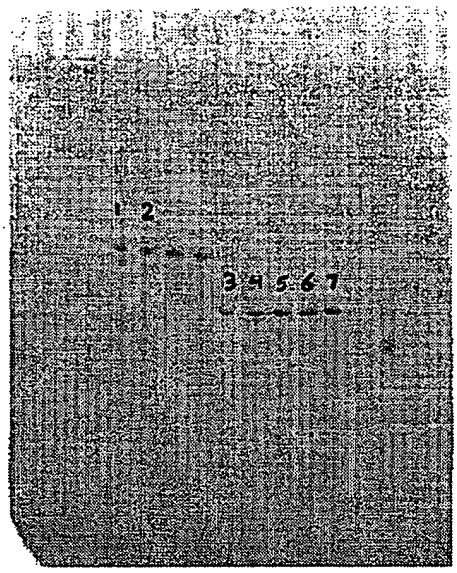

FIG. 11

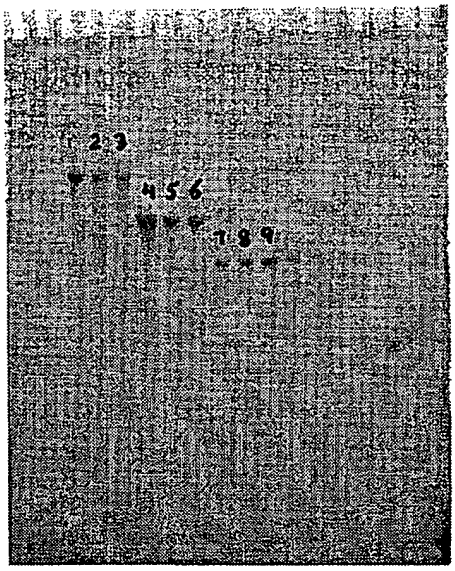

FIG.12

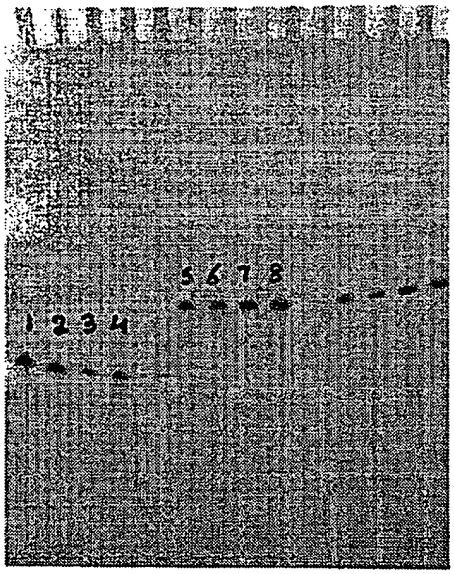

FIG.13

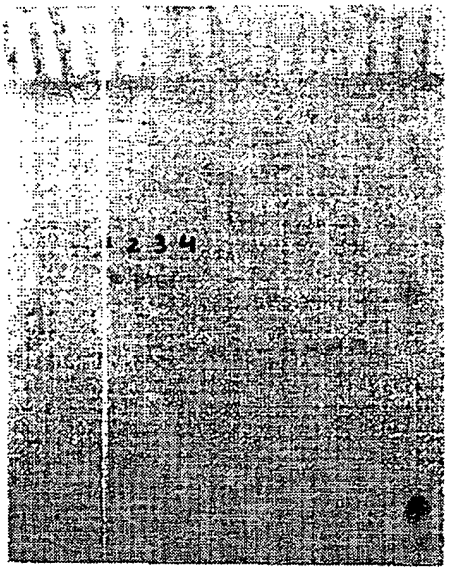

FIG. 14

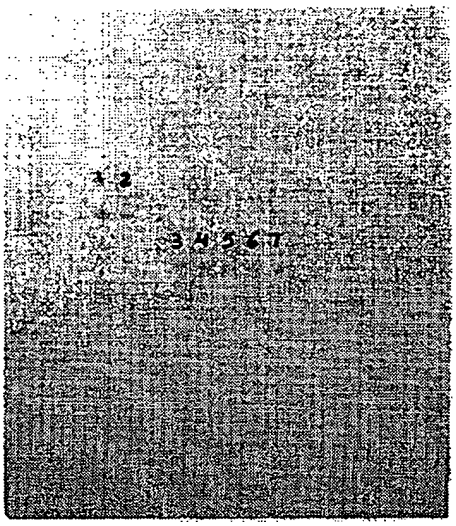

FIG. 15

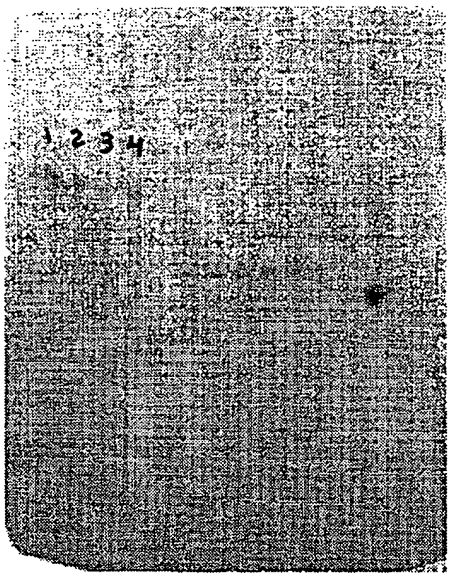

FIG. 16

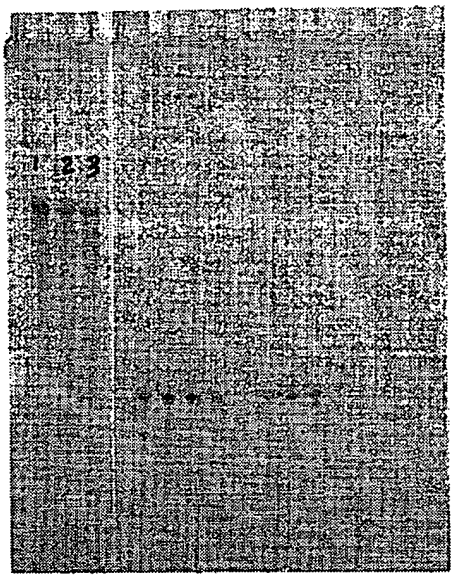

FIG.17

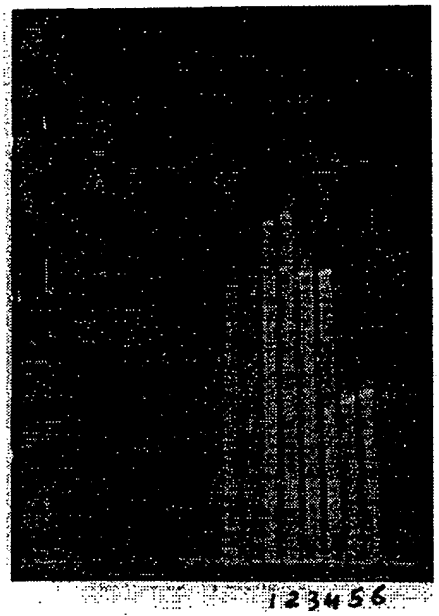

FIG. 18

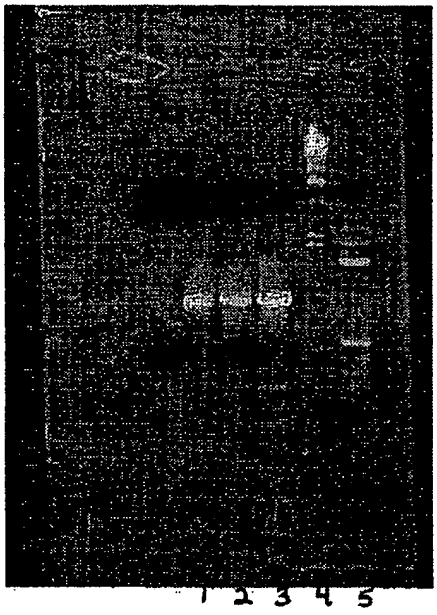

FIG. 19

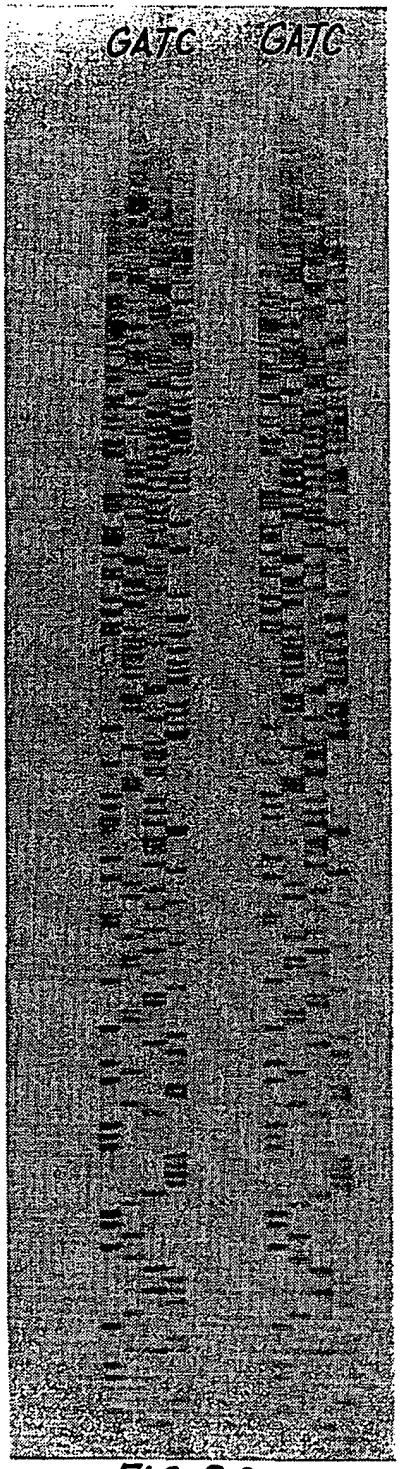

FIG. 20